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A KARYOLOGICAL STUDY ON THREE TAXA OF *SILENE* L. (CARYOPHYLLACEAE) BY USE OF AN IMAGE ANALYSIS SYSTEM

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ABSTRACT

Karyological analyses of three taxa of *Silene* (Caryophyllaceae) from Turkey were carried out in this study. Somatic chromosome numbers of 2n = 24 were determined for *S. lycaonica* and *S. duralii* and 2n = 48 for *S. cappadocica*. The basic chromosome numbers is x = 12 in all taxa. The karyotype analyses were studied by using an Image Analyzing System supported with computer. Also, karyograms and their idiograms were drawn.

KEY WORDS: Caryophyllaceae, *Silene*, Imaging techniques, karyotype, Turkey

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Silene (Caryophyllaceae) is one of the largest plant genera in the world with approximately 700 species, almost half of which grow in the Mediterranean region. Southwest Asia is one of the main centers of diversity for the genus, which is represented by 136 species in Turkey (Coode and Cullen, 1967; Davis et al., 1988; Tan and Vural, 2000; Duran and Menemen, 2003; Deniz and Düşen, 2004; Bağcı, unpub. data 2007).

Silene has been studied using taxonomical (Duran and Menemen, 2003; Deniz and Düşen, 2004), morphological (Yıldız, 2006), molecular (Sandbrink and Brederode, 1991; Popp and Oxelman, 2001), palynological (Yıldız, 2006), chemical (Larhsini et al., 2003;

Dötterl et al., 2005) and cytotaxonomical data (Kruckeberg, 1955; Morisset and Bozman, 1969; Abdel Bari, 1973; Yıldız and Çırpıcı, 1996; Široký et al., 2001; Yıldız and Gücel, 2006). However, karyotypic studies have not been published for *Silene lycaonica* and *S. duralii*.

The chromosome numbers of the genus *Silene* are reported as 2n = 20, 24 and 28 in 40 taxa in the Turkish Flora (Coode and Cullen, 1967; Davis et al., 1988; Özhatay et al., 2000).

In the present study, the chromosome number and karyotype of the species has been studied (except *S. cappadocica*) for the first time.

MATERIALS AND METHODS

Voucher specimens for the present study were obtained as follows:

Section: *Sclerocalycinea* Boiss.

Silene lycaonica Chawdh. Caespitose, glabrous, perennial. Stems erect c. 25 cm. Calyx 12-16 mm, Anthophore 6-7 mm. Capsule included in the calyx. Konya: Konya-Taşkent-Ermenek road, 5 kilometers away from Taşkent., steppe, 15.07.2004. *Ertuğrul 3175 & Bağcı.* This species is endemic to Turkey.

Section: *Macranthae* (Rohrb.) Chowdh.

Silene duralii Y. Bağcı A densely tufted aerial suffruticose perennial, flowering stems 22-34 cm tall, canescent below, viscid above Inflorescence in compound dichasia or in a widely branched compound dichasium, viscid, glaucous. Calyx (6)7-9 mm long, glabrous. Capsule mature, ovoid, exserted from the calyx, 6-11(-12) mm long. Anthophore glabrous, 1.5-2.0 mm long. Karaman: Ermenek-Kazancı, Sarıova plateau, Kartalkaya, mountain steppe, 1750-1770 m., 16.06.2006, *Bağcı 3476.* This species is endemic to Turkey.

Section: *Spergulifolia* Boiss.

Silene cappadocica Boiss & Heldr. Perennial, stems ascending to erect, retrorsely puberulent, 10-50 cm. high. Calyx 3-5 mm long in functionally female flowers, 5-11 mm long in hermaphrodite flowers. Anthophore 3-4 mm long. Capsule included in the calyx. Konya: Ermenek-Bucakışla, Yellibel aisle, Teke Çatı vicinity, 1610 m., 17.07.2004. *Ertuğrul 3291, Bağcı & Dural.*

All of the cytological observations were made on root tips, germinated on wet filter paper in Petri dishes. After germination, fresh root tips pretreated in α-mono-bromonaphthalene at 4°C for 16 hours, and then fixed with glacial acetic acid: absolute alcohol (1:3) 4°C for 24 hours. These were deposited in 70% ethanol at 4°C. The root tips were hydrolyzed in 1N HCl at room temperature for 12 minutes. Finally, they were squashed and stained in 2% aceto-orcein. Permanent slides were prepared using standard liquid nitrogen method. Karyotypes were determined using Image Analysis System (BsPro200) on a personal computer (Martin et al., 2006).

RESULTS AND DISCUSSION

Chromosome number and ploidy level

Both chromosome number and morphology are analyzed for the first time for *S. duralii* and *S. lycaonica*. Diploid chromosome numbers of 2n = 24 were determined for *S. lycaonica* and *S. duralii* (Figs. 1, 2); *S. cappadocica* was found to be tetraploid with 2n = 48. Karyogram and idiograms of these taxa (Figs. 4-5) were drawn by use of an Image Analysis System.

Chromosome length

Based on total length, the chromosomes were organized in decreasing order for each species. Arm ratios of each were found to be 1.88 for *S. lycaonica*, 1.31 for *S. duralii* and 1.38 for *S. cappadocica*. The absolute chromosome lengths were measured for each species and differences were seen. The smallest chromosomes were those in *S. cappadocica*, ranging between 1.31-3.02 μm. A similar chromosome

length (1.78-3.72 µm) was observed in *S. duralii*. Finally, *S. lycaonica* had the largest chromosomes, ranging between 2.30-5.31 µm (Table 1).

Genome length

Differences between chromosome lengths of the three species were correlated with their mean genome length. The genome lengths of each taxon are given in Table 1. The smallest genome length was that of *S. duralii* (28.88 µm) and the largest that of *S. cappadocica* (49.52 µm). *Silene lycaonica* showed an intermediate genome length value of 44.34 µm (Table 1).

Karyotype analyses

Differing karyotype formulas were seen only in *S. lycaonica* (which was 8m+3sm+1st) and in *S. duralii* (which was 11m+1sm); the tetraploid, *S. cappadocica* had a karyotype formula of 20m+4sm. The karyotypes of these species were similar, consiting of metacentric and submetacentric chromosomes; *S. lycaonica* differed by having one pair of subtelocentric chromosomes.

In this study, we observed chromosome numbers and morphology that were comparable to previous cytotaxonomic studies on the genus *Silene* (Yıldız & Çırpıcı, 1996). In their cytotaxonomic study of 19 *Silene* species, 15 species were diploids (2n = 24) and four species (including *S. cappadocica*) were tetraploids (2n= 48). In addition, the basic chromosome number was determined as x = 12 (Yıldız & Çırpıcı, 1996).

Chromosome study of the autotetraploid, *Silene latifolia,* revealed the chromosome number as 2n = 4x = 48, much as in *S. cappadocica* (Široký et al., 1999). Široký et al., (2001) reported on four species of *Silene* (*S. latifolia*, *S. vulgaris*, *S. pendula* and *S. chalcedonica*) with diploid numbers of 2n = 2x = 24, much as found in the present study.

Ghazanfar (1983) determined the karyotypes of 14 species belonging to sections *Siphonomorpha* and *Auriculatae* of *Silene.* In *Silene viridiflora* and *S. nodulosa* there are satellites on metaphase

chromosomes and in *S. boryi* and *S. vallesia* tetraploidy was observed. In the present study, satellites were not observed *Silene* but in *S. cappadocica* tetraploidy was observed as previously reported.

Abdel Bari (1973) made a karyotype study of 22 taxa of *Silene*. Two different basic chromosome numbers were revealed: x = 10 and x = 12. Tetraploidy was only found in *S. vulgaris* subsp. *macrocarpa* (2n = 48).

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Table 1. Chromosome comparison in the four studied taxa of *Silene*. AR: arm ratio; CI: centromeric index; THC: total length of haploid complement; M: metacentric; SM: submetacentric; ST: subtelocentric.

Species	2n	Chromosome sizes (μm)	AR	CI	THC (μm)	M	SM	ST
S. lycaonica	24	2.30-5.31	1.88	3.09	44.3	8	3	1
S. duralii	24	1.78-3.72	1.31	3.61	28.9	11	1	-
S. cappadocica	48	1.31-3.02	1.38	1.78	49.5	20	4	-

Figure 1. *Silene lycaonica* (2n = 24). Scale = 10 μm.

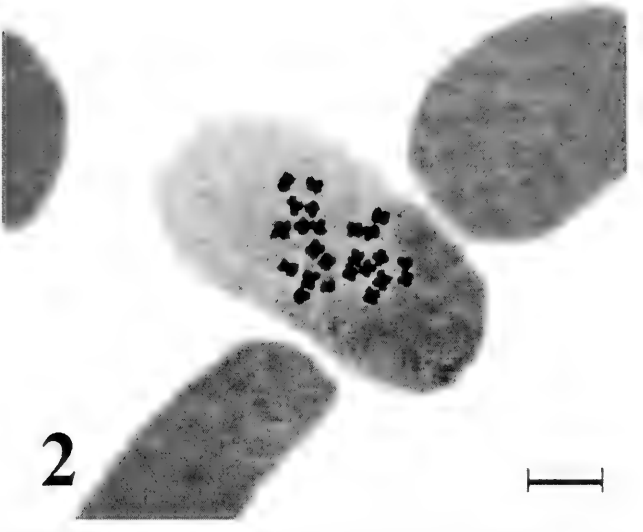


Figure 2. *S. duralii* (2n =24), 3. *S. cappadocica* (2n =48). Scale: 10 µm.

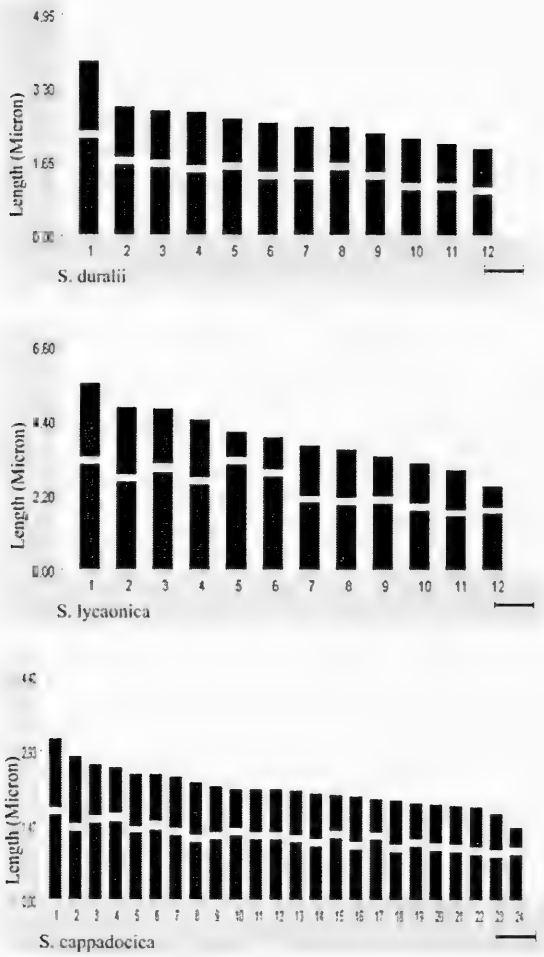
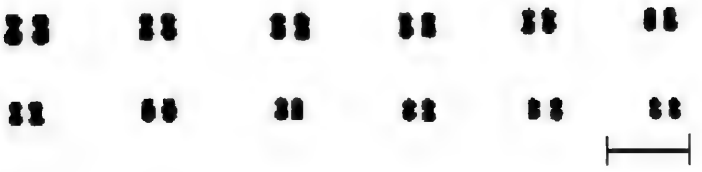
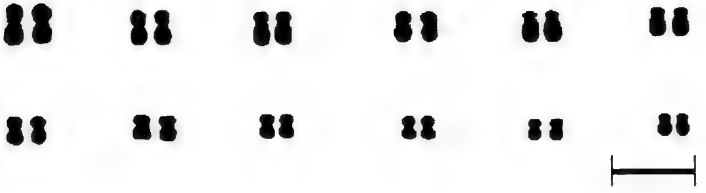


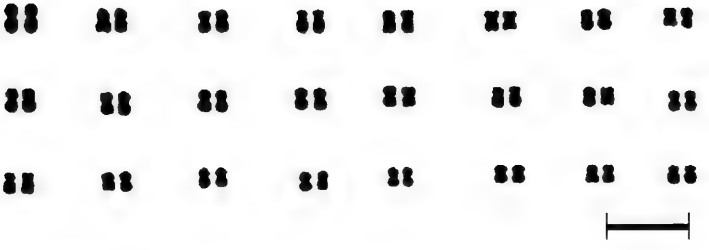
Figure 4. Idiograms of taxa belonging to *Silene*. Scale Bar: 10 μm.



S. lycaonica



S. duralii



S. cappadocica

Figure 5. Karyograms *Silene lycaonica*, *S. duralii* and *S. cappadocica*. Scale Bar: 10 μm.

SECALE CORNUTUM IS A FUNGAL CONDITION

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ABSTRACT

Secale cornutum, a name attributed to Nees but introduced into the literature earlier by Richard, would seem to be a name applicable to a species of the grass genus *Secale* (containing cultivated rye). However, examination of original literature and illustrations indicates that various authors involved were primarily attempting to discuss an ascomycetous fungus—what we now know to be *Claviceps purpurea* (ergot). After review of this information, it appears that use of the name *Secale cornutum* perhaps best served simply to describe the "spurred" appearance of cultivated rye spikes infected with ergot, i.e., exhibiting sclerotia or "ergot bodies." Fortunately perhaps, the name *Secale cornutum*, when apparently first formally introduced into the literature, was invalid.

KEY WORDS: Eclectic medical, ergot, infected, rye, sclerotia, *Secale cereale*.

The Eclectic Medical Movement in latter 19th century America—historically centered in Cincinnati, Ohio (Lloyd and Lloyd, 1910; Taylor, 1942)—emphasized specific botanical medicines, often with active alkaloid content. In pursuing some readings on this movement, I came across an unusual name for ergot, i.e., a name other than the correct Ascomycete name, *Claviceps purpurea* (Fries) Tulasne (or one of its fungal synonyms). Known for centuries as a plant disease (Davenport, 2005), ergot became especially noteworthy as the probable mind-altering agent (in contaminated rye cake) in the infamous Salem, Massachusetts witchcraft trials of the 1690s (Caporael, 1976). The ergot alkaloids are known not only to affect the nervous system, but to be powerful vasoconstrictors as well (Davenport, 2005). It makes sense, thus, that ergot was employed medicinally by Eclectic doctors (in the late 19th and early 20th centuries) as a spinal stimulant and,

particularly, to induce labor while controlling hemorrhage at the same time. In the writings of John Scudder (1870), a leading Eclectic physician and promoter of the "Eclectic School" in Ohio, the name for ergot is given as "*Secale cornutum*." This name also surfaced at a similar time in the literature of plant pathology, e.g., Rivolta (1873)—in the latter case *Secale* was misspelled as "Segala" (given as "Segala cornuta" in Rivolta's publication). Regardless of spelling, *Secale* is of course the genus name to which cultivated rye belongs, viz. *Secale cereale*. So, I wondered, were writers such as Scudder and Rivolta referring to ergot, unquestionably a fungus, as if it were a grass—as if it were a species of the genus to which cultivated rye belongs?

Use of the name *Secale cornutum* persisted for a time, seemingly in an accepted fashion. One may note, for example, the reference to ergot in *The Pharmacopoeia of the United States of America* (9th revision, 1916) as "*Secale cornutum*" or "spurred rye." The *Epitome of the Pharmacopeia of the United States and the National Formulary with Comments* (1943) mentioned *S. cornutum* as "ergot of rye," or "Ergota." Schiemann (1948) indicated *S. cornutum* to be a "drug" [from ergot], of special significance in obstetric applications. Reference to ergot, in drug form, as "secale cornutum" was apparently not uncommon in early 20th century pharmacopeias (Hatcher and Wilbert, 1915). However, confusion as to whether *S. cornutum* represented ergot, rye, "altered" rye, a possibly different species within the rye genus, a medication, or even something else, became apparent as I continued to explore such references. Obviously, some deeper detective work was in order.

In his scholarly index to botanical illustrations, Pritzel (1855) listed *Secale cornutum*, crediting Nees (1833) as the source of origin of the original illustration (Fig. 1) and, seemingly, the name as well. Pritzel correctly cited Nees' illustration #24 as applying to *S. cornutum*. *Index Kewensis* (original volumes) subsequently also listed *S. cornutum*, but mistakenly indicated Nees' illustration #74, rather than #24, as applicable to this "species." *Index Kewensis* attributed authorship of *S. cornutum* as "Offic. ex Nees"—however, "Offic." apparently refers to part of the title of Nees' publication (see Literature Cited, viz., "officineller"). *Index Kewensis* nonetheless appropriately equated the name *S. cornutum* to *Claviceps purpurea* (the correct name

for the ergot fungus), drawing attention to the awkward situation of the synonymy of a grass name and a fungal name. Chase and Niles (1963) gave the correct illustration (#24) for the Nees (1833) reference, but listed the 1866 edition of Pritzel's *Index*, rather than the 1855 edition in which the Nees reference was originally cited.

The only other authors mentioned by Pritzel (1855) in connection with *Secale cornutum*—these apparently given secondarily—are "Guimpel et Schl." ("Schl." = Schlechtendal). The Guimpel and Schlechtendal (1833) reference is contemporary with Nees (1833), and is not viewed as a prior publication. However, there are prior uses of the name *S. cornutum*. Nees himself (Nees & Ebermaier, 1830) employed the name three years prior to the reference (Nees, 1833) cited in Pritzel (1855). In the same year as Nees & Ebermaier, Geiger (1830) also gave *S. cornutum* as the name for "Mutterkorn" (ergot). The earliest mention of the name *S. cornutum* in the literature that I have found, however, is by Richard (1824). In Richard's (1824) work, *Secale cornutum* is listed as an "*altere namen*" for "*Sclerotium clavus*" (= *Claviceps purpurea*, cf. Oudemans, 1919; Walker, 1969). If Richard's (1824) work proves to be, as it seems, the earliest appearance of the name *Secale cornutum*, then its first formal usage would be as a synonym—and the name can be dismissed as invalid on this basis (Article 34, *International Code of Botanical Nomenclature*). The earliest reference indicated by Nees (1833) is in fact Richard (1824). Since Richard considered *S. cornutum* merely an "alternate name," one might wonder if some use of the name prior to Richard (1824) occurred in the literature. However, the French edition of Richard's work, appearing one year earlier (1823), does not include *Secale cornutum*. Perhaps any earlier use, i.e., before Richard (1824), was simply a part of oral tradition or correspondence. But, in any case, here the trail runs cold. Roshevitz (1947), in a monograph of *Secale*, did not pick up the name *S. cornutum*.

Although it is evident that Nees did not originate the name, the most substantial information available on *Secale cornutum* is still the Nees (1833) reference, complete with illustration (i.e., the reference selected by Pritzel, 1855, for citation). Pertinent here, however, is that it was not Nees' intent to describe a grass, but rather to describe a fungus. The fungus he was describing was of course ergot (*Claviceps*

purpurea), known then generally by the older name *Sphacelia segetum* (according to Hawksworth et al., 1983, the "teleomorph" of *Claviceps*—and to Kirk et al., 2001, as being "anamorphic *Claviceps*"). Nees (1833) was not describing the species *Sphacelia segetum* since, as he knew, both the genus *Sphacelia* and the species *S. segetum* had been previously described by Léveillé (1827). What has seemingly escaped attention is that Nees was attempting to describe a variety of *Sphacelia segetum*, viz. *Sphacelia segetum* var. α *secalis*. Nees equated his variety of *Sphacelia segetum* to former names—*Sclerotium clavus* and *Spermoedia clavus* (these representing *Claviceps* or possibly related anamorphic forms; cf. Seymour, 1929; Clements and Shear, 1931; Walker, 1969; Kirk et al, 2001)—and, as well, to *Secale cornutum*. Nees indicated, though, that these former names referred (descriptively) more to the changed appearance of the ovaries of the rye plant than, specifically, to the fungus itself—hence, his rationale for describing *Sphacelia segetum* var. α *secalis*—an apparent attempt to clarify the situation. Perplexingly, *Index Fungorum* lists "f. [forma] *secalis*," attributed with a "?" to "Krebs"—no date provided—but omits var. "α *secalis*" of Nees.

In any event, careful scrutiny of Nees' (1833) publication provides the answer sought. The name *Secale cornutum* was, albeit perhaps ill-advisedly, applied to the visible "condition" of cultivated rye grass when infected with ergot—a condition in which the dark, sometimes curved, beak-like sclerotia (resting bodies) of the ergot fungus have replaced the "grain" in a number of the grass florets. These "ergot bodies" are seen to occur intermittently—sometimes "spur-like" or "horn-like" in appearance—along the rye inflorescence (Fig. 1). The common name "ergot" is derived from the French *argot*, an allusion to the resemblance of the sclerotium to a "cock's spur" (Wolf and Wolf, 1947). The epithet "*cornutum*" means "horned," in reference to these same observed structures (sclerotia). The name *Secale cornutum* may, thus, be interpreted literally as "rye plants" seen to have these "horns" or "spurs"—in other words, rye plants contaminated or altered in this manner. Hence, the name *S. cornutum* has application, intent-wise at least, neither specifically as a species of rye, nor as a species of fungus—nor as a drug, for that matter. It refers primarily to the unusual physical appearance of the *infected* rye plant. As concerns nomenclature, we might nonetheless have to deal with the name (under

the rye genus, *Secale*, or the fungal genus, *Claviceps*) were it not for the fact that *S. cornutum*—based on knowledge at hand—may be viewed as an illegitimate name in either case, and disregarded as any sort of proper name. As discussed, the apparent initial appearance of the name in the literature was not taxonomically valid. Perhaps, designation as a *nomen invalidum* is, after all, the best nomenclatural repository for the name *Secale cornutum*.

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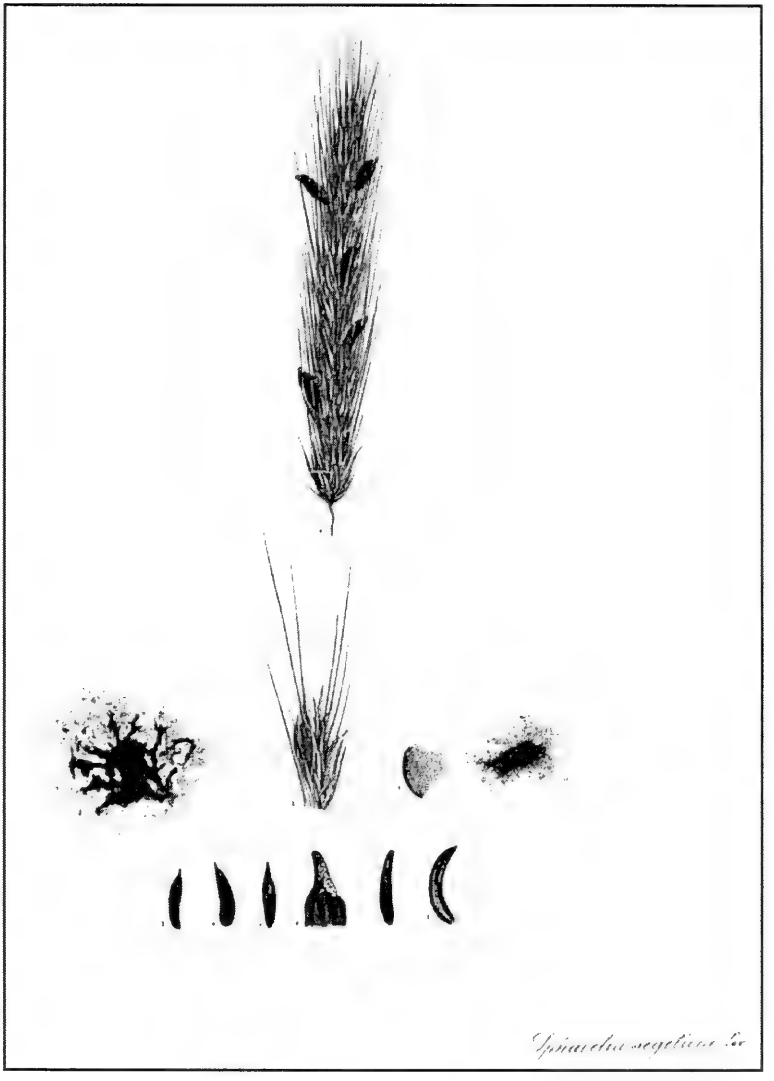

Fig. 1. *Sphacelia segetum* (alias *Secale cornutum*), from Nees (1833, illustration #24). We would now interpret this as *Claviceps purpurea* (ergot) on cultivated rye, *Secale cereale*.

TAXONOMIC REVIEW OF *SOLIDAGO PETIOLARIS* AND *S. WRIGHTII* (ASTERACEAE: ASTEREAE)

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ABSTRACT

Solidago petiolaris is treated to include three morpho-geographic entities. Plants east of the Mississippi River (Alabama, Florida, Georgia, South Carolina, North Carolina) are the typical variety; those west of the Mississippi (Louisiana, Arkansas, Texas, Oklahoma, Kansas, Nebraska, Missouri -- and including southern Illinois) comprise two additional taxa. Phyllaries of plants in the eastern portion of the western range (var. *angusta* [Torr. & Gray] A. Gray) are glandular and without other vestiture; phyllaries of plants in the western portion (var. *wardii* [Britt.] Fern.) are eglandular and finely strigose. *Solidago wrightii* is closely similar to *S. petiolaris*, especially where their ranges approach each other, and they show parallel trends of variation in leaf shape and involucral vestiture. Within *S. wrightii*, glandular plants apparently are populational variants without geographic coherence and are treated here as **S. wrightii** forma **adenophora** (Blake) Nesom, comb. et stat. nov. A narrow-leaved race near the southeastern corner of the range of the species (in Chaves Co. and Eddy Co., N.M., and Culberson Co., Tex.) is recognized as **S. wrightii** var. **guadalupensis** Nesom, var. nov. A collection by Charles Wright is selected as lectotype for *S. wrightii*.

KEY WORDS: *Solidago*, *S. petiolaris*, *S. wrightii* forma *adenophora*, *S. wrightii* var. *guadalupensis*, (ASTERACEAE: ASTEREAE)

Solidago petiolaris occurs primarily in two disjunct geographic segments. The typical plants are in the eastern range: Alabama, northern Florida, Georgia, South Carolina, and North Carolina. A disjunct and broader western range is west of the

Mississippi River (except for a few counties in southern Illinois): Louisiana, Arkansas, Texas, Oklahoma, Kansas, Nebraska, and Missouri. Another small area of disjunct populations occurs in northern Coahuila, Mexico (Sierra del Carmen, Sierra de la Encantada, Sierra del Jardin; Fig. 1). In an earlier survey of the species (Nesom 1990), I concluded that formal variants, previously recognized primarily on the basis of foliar features, were not justifiably recognized. The current set of observations, however, indicates that the previous study was short-sighted, as variants in vestiture clearly do occupy distinct regions within the western segment of the range.

Two expressions of phyllary vestiture occur in plants of the western segment of the species range. Those in the eastern half of the western segment have phyllaries with slightly raised glands, without other vestiture; the name *S. petiolaris* var. *angusta* (Torr. & Gray) A. Gray (type from Rapides Par., Louisiana) applies to these. Plants in the western half of the western segment have eglandular, finely strigose phyllaries; the name *S. petiolaris* var. *wardii* (Britt.) Fern. (type from Clark Co., Kansas) applies to these. Relatively few intermediates in vestiture occur where the two expressions meet or overlap.

Within the range of both var. *angusta* and var. *wardii*, phyllary vestiture often is suppressed and the phyllaries are glabrous or nearly so (without glands or without strigose hairs). Glabrous phyllaries of plants of var. *angusta*, however, often appear somewhat viscid. If only glabrous phyllaries have been observed for a particular county or parish, the map symbol for Fig. 1 is open.

In the original description of *Solidago angusta* Torr. & Gray (*S. petiolaris* var. *angusta*), the name apparently was intended to recognize plants with narrowly lanceolate to narrowly oblanceolate leaves (vs. the predominant lanceolate-elliptic to ovate-elliptic leaf shape). In some areas (e.g., Ouachita Par., La.) all plants of numerous collections produce the conspicuously narrow leaves; in other areas, more variability in leaf shape exists and intermediates are numerous, grading between narrower and broader forms. Counties and parishes in which distinctly narrow-leaved plants occur are indicated in Fig. 1, but in many places it is not possible to unarbitrarily distinguish taxa based on leaf shape. The name var. *angusta* can be used to refer to narrow-

leaved plants, but narrow-leaved plants occur within the range of those with glandular phyllaries, thus var. *angusta* also may refer to all of the latter, as here. A parallel trend in leaf width variation occurs in *S. wrightii* (see comments below).

Plants of the disjunct cluster of populations in Coahuila produce a relatively narrow capitulescence, glabrous achenes, and glandular involucres and they appear to be appropriately identified as *Solidago petiolaris* var. *angusta*, even though they are closer geographically to populations of *S. wrightii* than to the nearest *S. petiolaris*. The pattern of disjunction is similar to that in *Symphyotrichum drummondii* and *S. oolentangiense* (Nesom 1993), and *Solidago nemoralis* also now is known to have the same disjunction (Nesom in prep.). On the other hand, populations of *Symphyotrichum laeve* var. *geyeri* in the same region of northern Coahuila are disjunct from closest part of their main range in the Guadalupe Mountains of Culberson Co., Texas, and northward in New Mexico.

Plants of the southeastern U.S.A. (var. *petiolaris*) are variable in phyllary vestiture but geographical patterns of variation are not evident. Phyllaries vary from minutely stipitate-glandular without other vestiture to sparsely or moderately puberulent-hirsute and eglandular, or they may produce a mixture of glands and nonglandular hairs. Upper stems and leaves also may be glandular or eglandular and vary in density of nonglandular hairs.

Solidago petiolaris Ait., Hort. Kew. 3:216. 1789. *Aster petiolaris* (Aiton) Kuntze, Revis. Gen. Pl. 1: 318. 1891. Type: U.S.A. Aiton's entry for the species is this: "S. caule erecto villoso, foliis ellipticis scabricaulis petiolatis, racemis erectis, ligulis elongatis. Late-flowering Golden-rod. Nat. of North America. Cult. 1758, by Mr. Philip Miller. Fl. October-December. H. 4." (holotype: BM).

a. Solidago petiolaris var. **petiolaris**

Solidago milleriana Mackenzie in Small, Man. Southeast. Fl. 1350, 1509. 1933. Type: Mackenzie did not cite a type but noted (p. 1509) that *S. milleriana* was equivalent to "*Solidago petiolaris*

Authors, not Ait." Perhaps Mackenzie thought that Aiton's name had been misapplied, or perhaps he was proposing a new species for a southern segregate of *S. petiolaris*. A collection at NY is annotated in script (but apparently not in Miller's hand) as "*S. milleriana*" and may represent the name (South Carolina, *J.K. Small et al. s.n.*).

Solidago harperi Mackenzie in Small, Man. Southeast. Fl. 1352, 1509. 1933. Type: U.S.A. Georgia. Randolph Co.: dry woods near Grier's Cave, Midway (Lower Eocene) Geological Formation, 23 Oct 1902, *R.M. Harper 1778* (holotype: NY internet image!).

b. Solidago petiolaris var. **angusta** (Torr. & Gray) A. Gray, Proc. Amer. Acad. Arts 17: 189. 1882. *Solidago angusta* Torr. & Gray, Fl. N. Amer. 2: 204. 1842. Lectotype (Nesom 1990, p. 446): U.S.A. Louisiana. [Rapides Par.]: Alexandria, [no date, but ca. 1834–1840] *J. Hale s.n.* (NY! internet image; isolectotype: NY! internet image). Taylor and Taylor (1984, p. 238) cited the NY Hale collection as "holotype."

Solidago lindheimeriana Scheele, Linnaea 21: 599. 1848. *Aster lindheimerianus* (Scheele) Kuntze, Revis. Gen. Pl. 1: 318. 1891. Type: U.S.A. Texas. [Bexar or Comal Co.:] between New Braunfels and San Antonio, Oct 1848, *F.J. Lindheimer 417* (holotype: MO?; isotypes: GH!, US!).

c. Solidago petiolaris var. **wardii** (Britt.) Fern., Rhodora 10: 87. 1908. *Solidago wardii* Britt., Man. Fl. N. States 935. 1901. Type: U.S.A. Kansas. Clark Co.: 8 mi W of Ashland, 2 Oct 1897, *L.F. Ward s.n.* (holotype: NY! internet image; isotype: GH).

Variation in *Solidago wrightii*.

Solidago wrightii occurs from central Arizona through most of New Mexico, into southeastern Colorado, and into trans-Pecos Texas (Fig. 2). The range extends southward in Mexico into Sonora, Chihuahua, Durango, and southwestern Coahuila (Sierra de Jimulco).

Plants originally described as *Solidago wrightii* var. *orientalis* Nesom (Nesom 1989) were later segregated as specific rank (Nesom 1990). *Solidago orientalis* (Nesom) Nesom, of Coahuila and Nuevo León, is distinct from *S. wrightii* in its stems, leaves, and phyllaries with a mixture of stipitate glands and hirtellous vestiture, serrate (vs. mostly entire) leaves, heads in an interrupted, elongate capitulescence of axillary clusters, and fewer (4–6) ray florets.

Solidago wrightii also is closely related to *S. petiolaris*, the latter distinct in its combination of cylindric capitulescence, eglandular leaves, and glabrous or glabrate achenes. As noted below, however, achene vestiture and capitulescence shape vary in *S. wrightii*, and no single character provides consistently diagnostic distinction between the two taxa.

1. Plants caespitose from thick, woody bases, not rhizomatous; uppermost leaves often minutely glandular (lens); heads in more or less flat-topped or broadly rounded corymboid cymes or an open, paniculate cluster of corymboid cymes, sometimes in a narrow, subcylindric panicle; achenes strigose, rarely glabrous (in var. *guadalupensis*).
.....**Solidago wrightii**
1. Plants from a short caudex, often with a thickened, primary rhizome (commonly not evident in collections), production of scale-leaved rhizomes sometimes evident; leaves usually eglandular; heads usually in a narrow, subcylindric panicle; achenes glabrous or sparsely strigose at the summit.....**Solidago petiolaris**

Blake (1929) used the name *Solidago wrightii* var. *adenophora* to describe plants with stipitate-glandular vestiture (without non-glandular hairs) on the phyllaries and stems. Stipitate-glandular plants are the most commonly collected expression of the species and apparently occur over its whole range but without a geographic pattern that would evidence the evolutionary coherence of the taxon. Otherwise, plants are eglandular or minutely and inconspicuously glandular. Intermediates are not as abundant as might be expected, and this probably has contributed to the persistent use of varietal rank for glandular plants. Intermediacy does occur, however, most commonly in plants with glandular phyllaries but hirsute-puberulous (eglandular) stems. In others, phyllaries show a mixture of

glandular and non-glandular hairs. Taylor and Taylor (1983, 1984) noted that intergrading variation occurs in vestiture although they distinguished two varieties based on capitulescence shape. Semple and Cook (2006) did not recognize glandular plants of *S. wrightii* with formal nomenclature, noting intergradation in vestiture.

In some parts of the range of *Solidago wrightii*, one vestiture form or another appears to be the predominant expression. For example, in the White Mountains of Lincoln and Otero counties, New Mexico, 13 of 14 collections studied have glandular stems and phyllaries; in the area of Grant-Catron-Sierra counties, both expressions occur commonly but 5 of 5 collections from Hillsboro Peak (Sierra Co.) are glandular. In Brewster Co., Texas, apparently all plants have glandular stems and phyllaries, but in adjacent Jeff Davis Co. (the type locality for the species), stems and phyllaries are hairy and essentially eglandular. Eglandular plants are uncommon in the Mexican range. In New Mexico, glandular plants have been collected at an elevational range of (6000–)6600–10,400 feet, while eglandular ones have been collected at (5650–)6800–9200 feet. Since the variability in vestiture seems to be a populational phenomenon, glandular variants are recognized here at the rank of forma, rather than variety.

A distinctive narrow-leaved race of *Solidago wrightii* is endemic to the Guadalupe Mountains of northwestern Culberson Co., Texas, and adjacent Eddy Co., New Mexico, and apparently disjunct slightly northward to the east flank of the Sacramento Mountains in Chaves Co., New Mexico (Figs. 2, 3). These variants are recognized here as *S. wrightii* var. *guadalupensis*. The plants occur over limestone substrate, similar to habitats of var. *wrightii* in the Sacramento Mountains, but southward in Texas, *S. wrightii* occurs only in the volcanic Davis Mountains (Jeff Davis Co.) and on igneous "plugs" in the predominantly limestone Del Norte Mts. and Glass Mts. (both in Brewster Co.). The narrow-leaved plants occur at lower elevations than typical *S. wrightii* in New Mexico (4800–7100 ft vs. [5650–]6600–10,400 ft), but populations of var. *wrightii* in Jeff Davis Co. occur at similarly low elevation.

The populations of *Solidago wrightii* in Jeff Davis and Brewster Co. are disjunct and ecologically distinct from those in the

broader contiguous range of the typical expression, and it would not be surprising if the trans-Pecos populations were more closely related cladistically to var. *guadalupensis* than to the rest of var. *wrightii*. Morphologically, however, the similarity of the Texas plants of var. *wrightii* to those in the broader range is taken here as justifying their convarietal treatment. Assignment of rank to the geographically juxtaposed var. *guadalupensis* is equally problematic -- it might with justification be considered a weakly differentiated species.

Var. *guadalupensis* is morphologically discontinuous from populations of var. *wrightii*, but the former produces nearly glabrous achenes, similar to those in *S. petiolaris*. Collections of *Solidago wrightii* in southeastern Colorado and eastern New Mexico (including Harding, Quay, and Union counties) have glabrous achenes and relatively elongate capitulescences (also similar to *S. petiolaris*), but the plants have glandular phyllaries, stems, and leaf surfaces. *Fletcher 5800* (UNM) from Harding Co. and *Williams s.n.* (UNM) from Quay Co. have minutely glandular leaf surfaces, hirsute-glandular involucres, and are similar in overall habit to others of var. *wrightii* from northern New Mexico.

The closest documented geographical approach of *Solidago wrightii* to *S. petiolaris* are plants of the latter in Cimarron Co., Oklahoma (*Taylor 23743*-BRIT, *Waterfall 9728*-SMU; Fig. 1) and others in the Texas panhandle, which have eglandular, villous-strigose phyllaries typical of *S. petiolaris* var. *wardii*. Thus, even though consistent diagnostic characters do not separate the two species and trends in variation in vestiture and leaf shape in *S. wrightii* are paralleled by similar trends in *S. petiolaris*, each taxon has a geographic coherence and the two are distinct at their point of near-contiguity in range.

Solidago wrightii A. Gray, Proc. Amer. Acad. Arts 16: 80. 1881. *Solidago bigelovii* var. *wrightii* (A. Gray) A. Gray, Proc. Amer. Acad. Arts 17:190. 1882. Lectotype (designated here): U.S.A. New Mexico, "collected in expedition from western Texas to El Paso, New Mexico, May-Oct 1849, *C. Wright 281* (GH; isolectotype: US internet image!). In the protologue, Gray cited "*S. petiolaris*, var., Gray Pl. Wright. 1. 94. *S.*

californica, var., Rothrock, in Wheeler Rep., vi. 145. – W. Texas to Arizona, Wright, Bigelow, Rothrock." The entry in Plantae Wrightianae is "281. SOLIDAGO PETIOLARIS, Ait.; Torr. & Gray, Fl. 2. p. 203; var. Mountains between the Limpia and the Rio Grande; Aug." The entry in the Wheeler Report notes that "This form (730) from Mount Graham, Arizona, and at an altitude of 9,000 feet, may prove a distinct species."

Many collections of *Solidago wrightii* have been made in Texas from the Davis Mountains (Jeff Davis Co.) and from the Guadalupe Mountains (northwestern Culberson Co.); elsewhere in the state, it has been collected only from the Del Norte Mountains and Glass Mountains (Brewster Co.). Charles Wright collected in Jeff Davis Co. and it is probable that the type collection was made there. Shaw (1987) described the 1849 collecting trip of Wright, who accompanied an army wagon train under the command of Captain S.G. French. The group skirted the eastern margin of the Davis Mountains, passing through Limpia Canyon (present-day Hwy 17) and collecting further in the area of "Wild Rose Pass" of the canyon, about 10 miles northeast of Fort Davis. From there, they passed around the southern end of the Davis mountains and continued west to Chispa Mountain and the nearby settlement of Lobo in southernmost Culberson County. Collections variously labeled "mountains west of Limpia Pass," "mountains beyond the Limpia," and "hills between Limpia and Rio Grande," were made on 26-28 August at the southern end of the Davis Mountains (Geiser 1935). Wright's field notes indicate that he made three collections of Compositae at the southern end of the Davis Mountains (Johnston 1940): *1034* (Wright's field number), "Mt. valleys beyond Limpia Pass," 26 Aug 1849; *1043*, "hills beyond Limpia Pass," 27 Aug 1849; and *1055*, "mountains beyond Limpia Pass, dense bunches or branched from the root. fl. yellow," 28 Aug 1849. Wright's field number apparently is not associated with the lectotype, but it seems likely that 1055 was his collection of the goldenrod; Asa Gray assigned the number "281" to the collection.

Solidago bigelovii A. Gray, Proc. Amer. Acad. Arts 16: 80. 1881. *Aster brittonii* Kuntze, Revis. Gen. Pl. 1: 315. 1891 [nom. nov., based on *Solidago bigelovii* A. Gray, non *Aster bigelovii* A. Gray]. Type: U.S.A. New Mexico. [Grant Co.]: Copper mines, 1851, *C. Wright 1182* (holotype: GH; isotypes: PH, US internet image!). In the protologue, Gray noted that "*S. bigelovii* is a New Mexican species founded on *S. petiolaris*, var., Gray in Bot. Mex. Bound. 79, collected by Bigelow, Wright, and Parry ... ." The entry in the Boundary Survey is "SOLIDAGO PETIOLARIS, Ait.; Torr. & Gray, l.c. Cobre, New Mexico, etc."

Solidago wrightii forma **adenophora** (Blake) Nesom, comb. et stat. nov. Based on *Solidago wrightii* var. *adenophora* Blake, J. Wash. Acad. Sci. 19: 269. 1929. Type: U.S.A. Arizona. Mt. Lemmon, 7500 ft, 4 Sep 1926, *G.J. Harrison 3016* (holotype: US! internet image!).

Solidago wrightii var. **guadalupensis** Nesom, var. nov. Type: U.S.A. Texas. Culberson Co.: Guadalupe Mts., south McKittrick Canyon, 27 Sep 1962, *D.S. Correll 26048* (holotype: LL!; isotype: SMU!).

A *Solidagini wrightii* typico similis involucris glandulosis sed differt foliis lanceolatis vel lineari-lanceolatis et acheniis glabratis.

Upper stems sparsely to densely hirsutulous-puberulent to puberulent, eglandular. Leaves mostly lanceolate, midstem 3–6 cm long, 4–7 mm wide, narrowly lanceolate to linear-lanceolate on distal 1/3, sparse and bracteate immediately below and into capitulescence. Capitulescence subcorymboid. Phyllaries glabrous to minutely sessile-glandular, without other vestiture, usually viscid when eglandular. Achenes glabrous (mostly) to sparsely strigose (rarely, e.g., *Correll 13869*).

Flowering (May–)Jun–Aug(–Sep); 4800–7100 feet elevation; cliff crevices, slopes and ridges, mine tailings, canyon bottoms, gravel alluvium of stream beds, always over limestone, in vegetation of acacia-juniper-dasylirion-lechuguilla, oak, oak-maple, and yellow pine-maple-hophornbeam-madrone.

Additional collections examined: **New Mexico. Chaves Co.**: E side of Sacramento Mts., 1 mi NE of windmill in Mule Canyon, 6200 ft, 25 Jul 1979, *Fletcher 3994* (UNM); E side of Sacramento Mts., cracks of E facing limestone scarp, Center Sec 4 T16S R16E, 6700 ft, 17 Jun 1981, *Fletcher 5205* (UNM). **Eddy Co.**: Carlsbad Caverns Natl. Monument, Hayhurst Ridge N, shoulder of sideslope, limestone, oak, 5855 ft, 15 Sep 2000, *Arbetan PA022-F3* (UNM); Guadalupe Mts., Lincoln National Forest, Devils Den Canyon, S to the end of rim road 540, then 2 mi SSW on Forest trail 202, mine tailings on NNW-facing slopes, oak, ca. 7200 ft, 6 Sep 1986, *Brunt 9* (NMC); Carlsbad Caverns Natl. Monument, Hayhurst NW, head of drainage (narrow canyon), limestone, oak-maple, 6155 ft, 14 Sep 2000, *Chauvin YC049-F1* (UNM); 44 mi SW of Carlsbad, Big Canyon, South Fork, 5200 ft, 28 Sep 1989, *Dunmire s.n.* (UNM); Guadalupe Mts., 12 Jul 1939, *Hershey s.n.* (NMC); E slope of Guadalupe Mts. about 48 air km SW of Carlsbad at Sitting Bull Falls, reached by NM Hwy 137, canyon bottom, limestone, *Mimosa*, *Juniperus*, *Dasylirion*, lechuguilla, *Juglans* in canyon, 1340 m, 21 Jun 1976, *Spellenberg 4199* (NMC); Guadalupe Mts., canyon system below and to W, NW, and E of Devil's Den Spring, 6900–7100 ft, 13 Jul 2000, *Worthington 30161* (UNM); Carlsbad Caverns Natl. Monument, Hayhurst Ridge N, limestone, oak, 5714 ft, 16 Sep 2000, *Yanoff SY014-F1* (UNM). **Texas. Culberson Co.**, Guadalupe Mts.: south McKittrick Canyon, frequent on canyon floor in open yellow pine, maple, Ostrya, madrone woodland, 4800 ft, 14 Sep 1978, *Anderson 4652* (BRIT); McKittrick Canyon, on rocks near stream, 18 Jul 1984, *Brown 7757* (BRIT); S of McKittrick Canyon, 2.4 km S, 1.0 km W Pratt Lodge, ca 1/2 km SW of Turtle Rock, open gravel alluvium, 5500 ft, 9 Sep 1975, *Burgess 3710* (TEX); stony mt. sides, 8 Aug 1931, *Clark 4267* (UNM); along mountain stream above Pine Spring Camp, 15 Aug 1946, *Correll 13869* (SMU); north McKittrick Canyon, gravelly stream bed, 18 Aug 1946, *Correll 13948* (LL, SMU); south fork of McKittrick Canyon, 21 Jun 1964, *Correll & Hanson 29808* (LL); mouth of McKittrick Canyon, along stream, 2 Jul 1958, *Correll & Johnston 19150* (LL); Pine Springs Canyon, 7 Sep 1961, *Correll & Johnston 24275* (LL); Pine Sping Canyon, 6800 ft, 2 Jun 1949, *Hinckley & Hinckley 12* (LL); above Frijole, canyon near spring, 9 Aug 1945, *Lundell 14399* (SMU); above McKittrick Canyon, open rocky ridge, 25 Jul 1931, *Moore & Steyermark 3629* (LL); Smith Canyon, shaded arroyos, 17 Jul 1945,

Muller 8297 (SMU); Smith Canyon infrequent in limestone crevices, 6200 ft, 10 Jul 1949, *Turner 1261* (SMU); lower McKittrick Canyon below Pratt Lodge, limestone soil, 5000 ft, 30 Aug 1950, *Warnock 9420* (SMU); south McKittrick Canyon, limestone soil, 5500 ft, 3 Aug 1952, *Warnock 10972* (LL, SMU); south rim of Pine Springs Canyon, on boulders, 23 Sep 1946, *Whitehouse 17114* (SMU-2 sheets).

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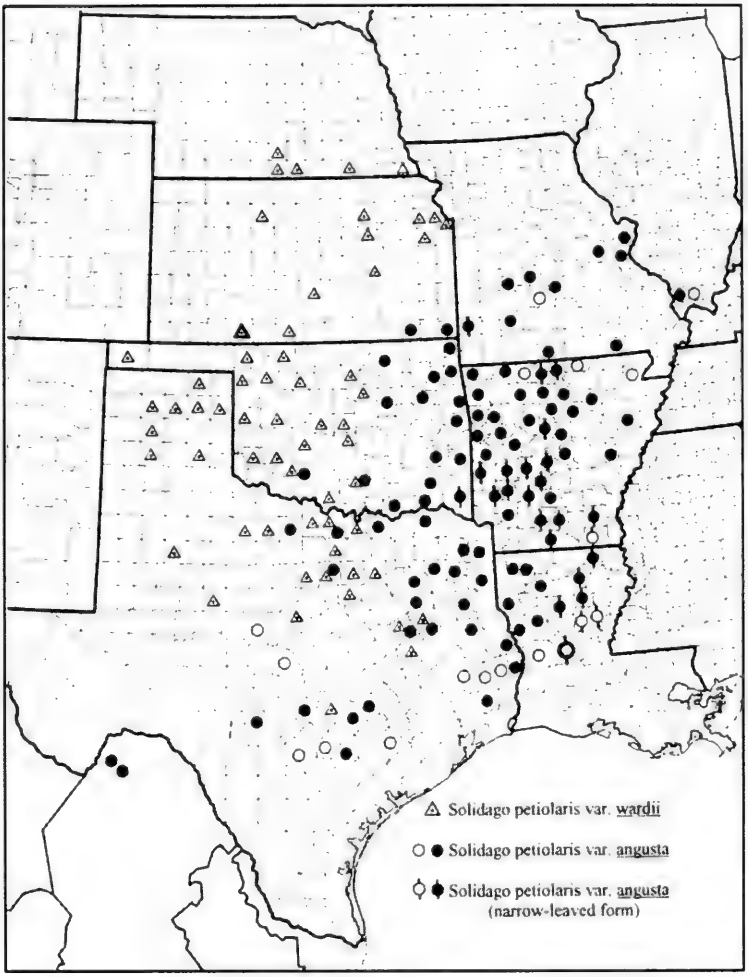

Figure 1. Western distribution of *Solidago petiolaris*: var. *angusta* and var. *wardii*. Bold symbols indicate type localities for the two varieties.

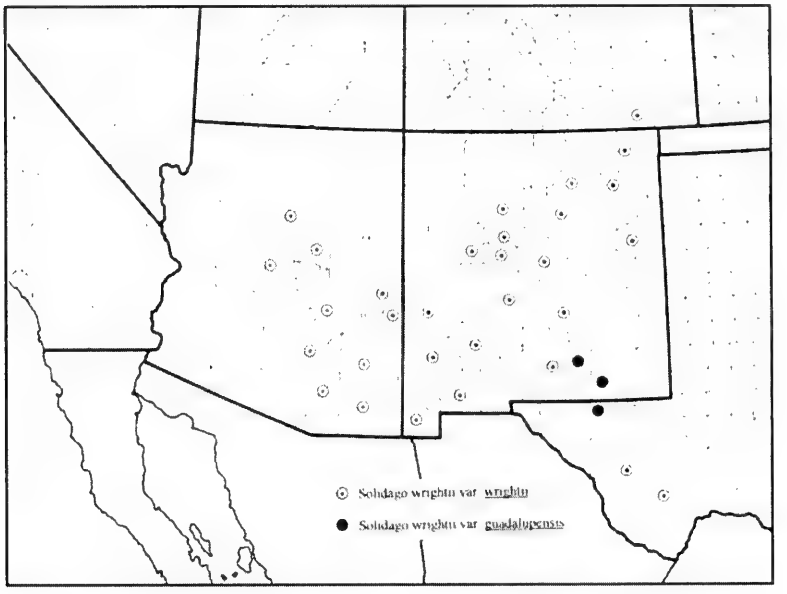

Figure 2. County-level distribution of *Solidago wrightii* in the U.S.A. The distribution continues southward into Mexico (see text).

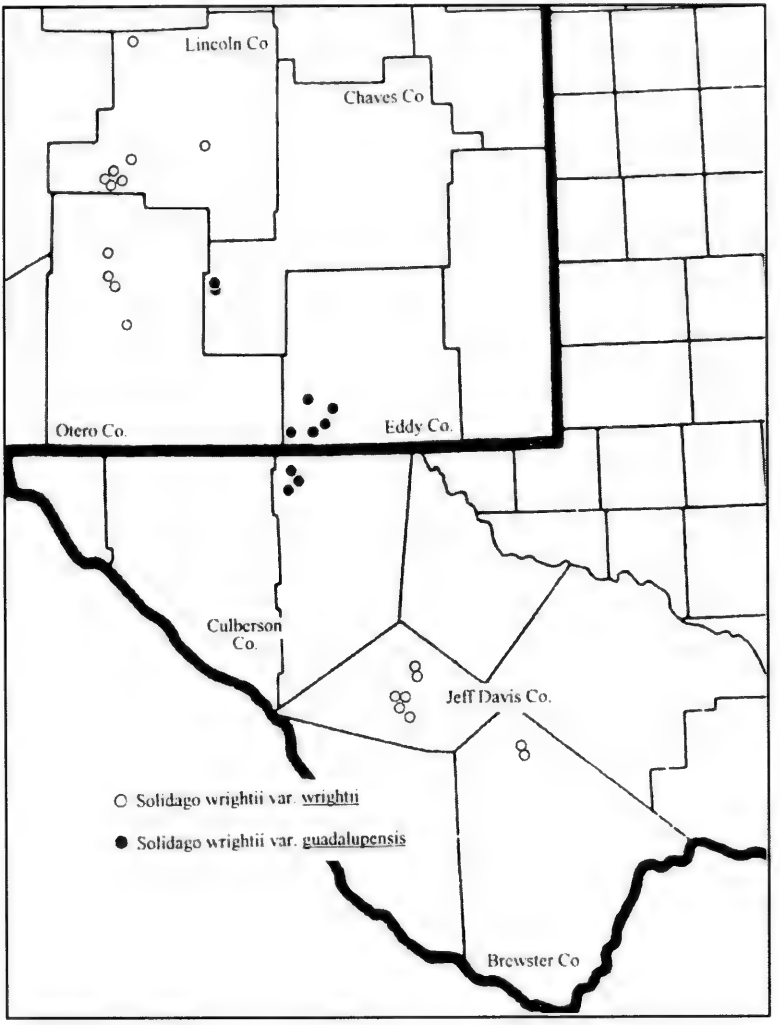

Figure 3. Detailed distribution of *Solidago wrightii* var. *guadalupensis* and closest populations of var. *wrightii*.

BIOLOGICAL STATUS OF *FUNASTRUM CYNANCHOIDES* AND *F. HARTWEGII* (ASCLEPIDACEAE)

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ABSTRACT

Funastrum cynanchoides and *F. hartwegii* have been treated as belonging to various genera, either as distinct species, subspecies and/or varieties of a single species. The nomenclatural history of the two taxa is reviewed, and it is concluded that they are best treated as distinct species; maps showing the distribution of both are provided.

KEY WORDS: Asclepiadaceae, *Funastrum*, Mexico, southwestern U.S.A.

The nomenclatural history of *Funastrum cynanchoides* Decne. is adequately reviewed by Holm (1950). Suffice to say that the taxon has been variously treated in three genera, either as a widespread variable species, or as composed of one or more infraspecific taxa.

Recent DNA data (Liede 1996) strongly suggest that the species is best placed in the genus *Funastrum*, as well-noted by Krings (2000).

Regarding the heterogeneity of *F. cynanchoides*, most recent workers have followed the treatment of Holm (1950), who recognized two partially sympatric subspecies in the group (subsp. *cynanchoides*, typified by material from northeastern Mexico, and subsp. *hartwegii* [Vail] R. Holm, typified by collections from northcentral Mexico), noting however, that they were well-marked, flowered at different times, and presumably formed the occasional hybrid. Holm did not recognize the varietal category, as some consider appropriate (Turner

and Nesom 2002). Krings (2000) corrected this oversight with his creation of the var. *hartwegii* (Vail) Krings.

But the biological problem remains: should the two infraspecific taxa be treated as but varietally distinct, or as species? The present paper addresses that question, this ignored by Krings.

Sarcostemma cynanchoides and its segregate, *S. hartwegii* (Vail) Schltr., were both recognized at the specific level by Schlechter (1914). Nevertheless, Holm, as already noted, treated these as but subspecies, remarking as how "Intergradation between the two forms appears to be uncommon, but occurs in a narrow northwest-southwest belt from Arizona to central Mexico. Hybridization has produced an abruptly stepped cline and intermediate forms have been designated as *S. c.* cl. *cynanchoides-Hartwegii*."

Holm goes on to list five hypothetical hybrids between the two taxa, most of which I have examined, and these might indeed be first-generation hybrids, but any evident backcrossing from such hybrids seem not to be apparent, nor does the large number of specimens examined by the present author from throughout the distribution of the taxa suggest that such occurs, nor evident clines derived from these. In short, the two taxa appear to be biological species that might occasionally form hybrids, this in spite of their different flowering times (*F. hartwegii* flowering in the spring; *F. cynanchoides* flowering in the late summer and fall).

The two species can be readily distinguished by the following key:

1. Leaves broadly lanceolate, 2-4 times as long as broad, cordate at the base; flowers mostly white; corona vesicles widest above the middle.....**F. cynanchoides**

1. Leaves narrowly lanceolate, 5-10 times as long as broad; flowers purple or pinkish; corona vesicles widest below the middle.....
.....**F. hartwegii**

In my Atlas of the Flora of Texas (Turner et al. 2003) I reluctantly treated the two taxa as but varieties, but subsequent study, both in the herbarium and in the field, has convinced me that the two taxa are valid biological species (sensu Mayr [1992], and others). Distributions of the two species are shown in figures 1 and 2, the data concerned based upon specimens cited by Holm and those on file at SRSC, LL, and TEX. It should be noted that *F. hartwegii*, while partially sympatric with *F. cynanchoides* in the southwestern U.S. and northern Mexico, does not normally occur with the latter, at least in Trans-Pecos, Texas. However, both may be found growing in relatively close proximity, *F. cynanchoides* usually in loose sandy soils; *F. hartwegii* in heavier calcareous soils. Most of the inferred populations are composed of either one species or the other, but it is likely that occasional hybrids do occur, as suggested by Holm in her herbarium analysis.

ACKNOWLEDGEMENTS

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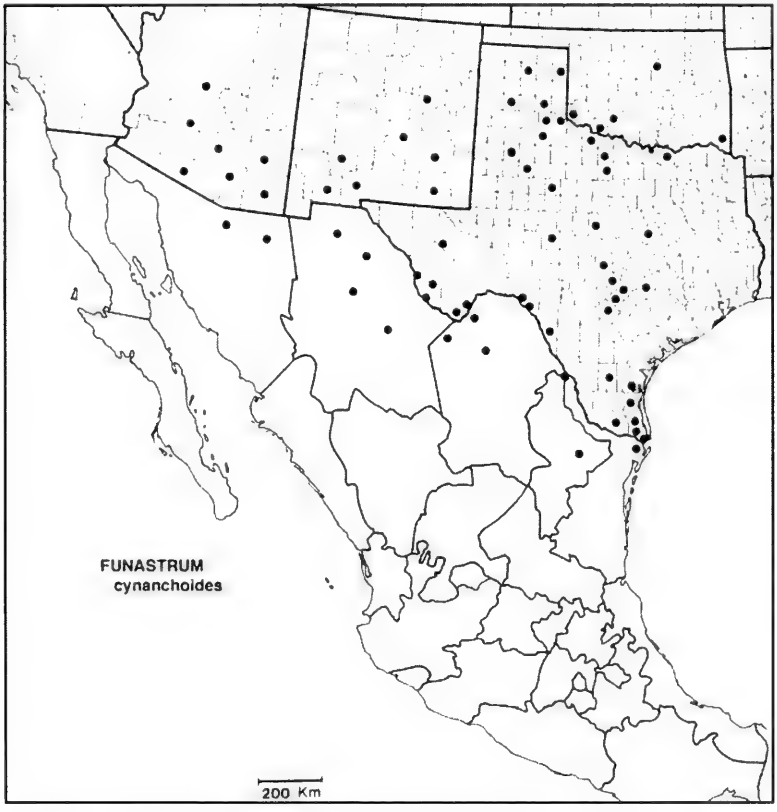

Figure 1. Distribution of *Funastrum cynanchoides*.

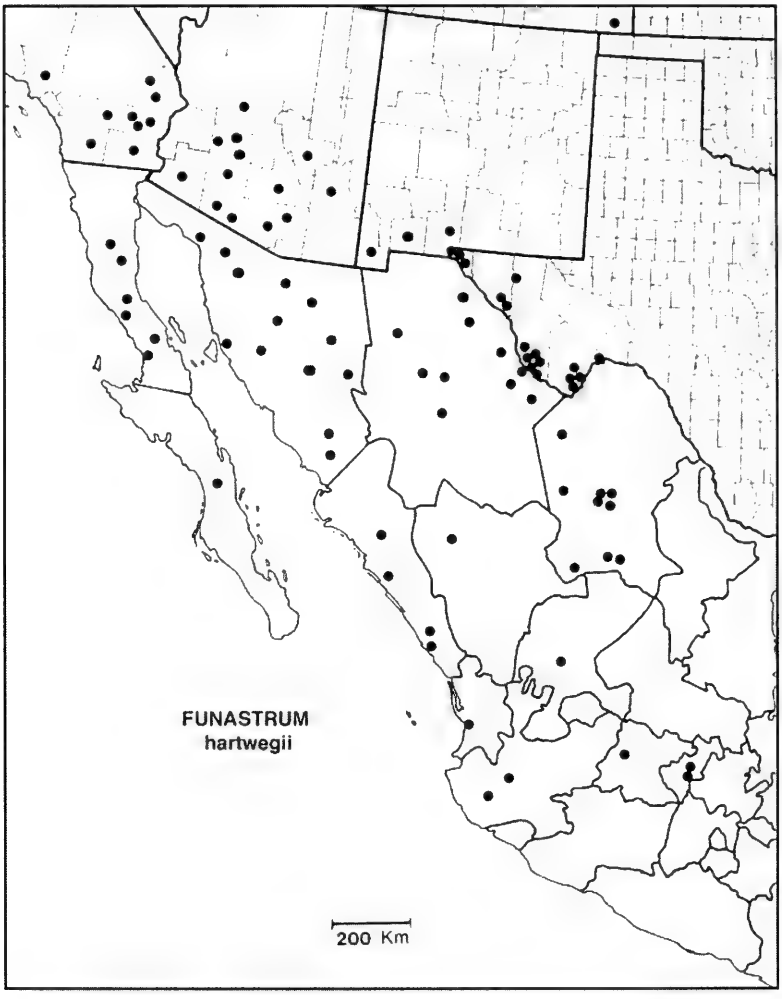

Figure 2. Distribution of *Funastrum hartwegii.*

KEYS TO THE FLORA OF FLORIDA: 18, *KALANCHOE* (CRASSULACEAE)

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ABSTRACT

Kalanchoe (Crassulaceae) is represented in Florida by 6 species and 1 self-sustaining hybrid, all restricted to the peninsula. The species are all introduced, while the hybrid (*K. houghtonii*) may be formed wherever *K. daigremontiana* and *K. delagoensis* occur together. An additional 5 species have been reported for the state, but are believed either not to persist outside of cultivation or are based on misidentifications. An amplified key is given to the Florida taxa.

KEY WORDS: *Kalanchoe*, Crassulaceae, Florida flora.

The genus *Kalanchoe* (Crassulaceae) has long been familiar in North American horticulture by the popularity of *K. pinnata*, the "air plant" or *Bryophyllum*, as a botanical novelty; the leaves may be pinned to window curtains so that rootlets, then miniature plantlets, develop at notches along the margin. This trait of vivipary is widespread within the genus, at times the primary mode of reproduction. All species are succulent, and typically shift under stress from the conventional C3 metabolism to the crassulacean acid C4 pathway as do many otherwise-dissimilar water-retentive plants.

All Florida species of *Kalanchoe* are native to South Africa or Madagascar, home of perhaps 125 diverse forms. This diversity has led to introduction elsewhere of many species (45 recorded in Europe, perhaps fewer in America) as horticultural novelties. Relatively few as yet have come into Florida, with many of those restricted to greenhouses and tropical gardens and not yet established outside of cultivation. As is invariably true of plants grown for novelty, levels of popularity rise and fall. *Kalanchoe pinnata*, once widely available for

purchase, is now so ubiquitous in greenhouses and naturalized where weather permits that its novelty and marketability is diminished. *Kalanchoe daigremontiana* and *K. delagoensis* have followed a similar trajectory. These species have been replaced by *Kalanchoe blossfeldiana*, now widely marketed as a popular houseplant.

Kalanchoe daigremontiana and *K. delagoensis*, both native to Madagascar but largely allopatric, when together in greenhouses in Europe and America appear to be the parents of a very common hybrid. For decades this plant has been recognized only as *Kalanchoe "aff Hybrida"* or some similar designation (Handbook of Succulent Plants 2: 650. 1978; Exotica, ser. IV. 1: 903. 1982; European Garden Flora 4: 181. 1995). It was recognized and well described by Arthur D. Houghton in 1935 (Cactus & Succulent Jour. 7: 44), but was not named. It has now been formally named *Kalanchoe houghtonii* (Cactus & Succulent Jour. 78: 92-05. 2006).

Kalanchoe houghtonii reproduces apparently exclusively by plantlets borne in the notches along leaf margins. Every notch of every leaf seemingly produces a plantlet, but never does a second form after the first has dropped. In suitable open sandy locations these multitudinous plantlets quickly form dense stands, and frequently become nuisances in flower beds and beneath greenhouse benches.

The monocarpic behavior of these three taxa is seldom recognized or understood. Grown from viviparous plantlets (or possibly from seeds in the case of the two putative parents), the plants develop vegetatively for several years. The stems remain unbranched; they often become unstable with increasing weight of successively larger leaves, toppling, then the apex turning to form a continuing upright stem. After perhaps 3 to 7 years the plant forms a robust terminal inflorescence; the entire plant then dies. This trait seems rare or is perhaps absent elsewhere in the genus.

Kalanchoe is here transcribed without the diaeresis over the terminal letter, though that sign is permitted (I.C.B.N., Art. 60.6) for those who feel need for guidance in pronunciation.

It is not surprising that species of *Kalanchoe* are not infrequently misidentified. A repeated pattern has been for a specimen to be misnamed; then later workers, lacking adequate alternative means of naming the plant, pick up the misidentification and disseminate it in their own publications. For many years the only readily available key to species in North American cultivation was by J. T. Baldwin (Amer. J. Bot. 25: 572-579. 1938). Perhaps even today the most useful treatment is that of H. R. Tolken (Flora of Southern Africa 14: 61-73. 1985), though his key necessarily lacks most Madagascarean species and includes many not found in North America. The present key is itself restricted to species known outside of cultivation, thus must be deficient in omitting those that are in the state but yet to be confirmed as escapes.

KALANCHOE Adans. [1]

1. Leaves pinnately compound on lower stem (simple above), fleshy but plane, broadly elliptic with crenate margin; flowers pendent; sepals long-connate, the calyx inflated; petals dusky rose, 2.5-3.5 cm. long. Fleshy perennial herb to 1.5 m. Coastal shell mounds, calcareous tropical hammocks. South and central peninsula (n. to Sarasota, Brevard counties); infrequent. Winter-spring. Somewhat invasive. An old dime-store favorite, the single leaves to be attached to window curtains where a small plantlet will form at each crenation. [*Bryophyllum pinnatum* (Lam.) Kurz]
BRYOPHYLLUM. * ***Kalanchoe pinnata*** (Lam.) Pers.

1. Leaves wholly simple (rarely some leaves pinnate in *K. crenata*), often very fleshy, plane or in some nearly as thick as broad; sepals free to short-connate.

    2. Plants branched, perennial, usually surviving and flowering for several seasons; leaves crenate, broadly elliptic to obovate, without flange at sinus, usually not plantlet-bearing; flowers erect or pendent.

3. Leaves openly spaced along stem, wholly green; flowers erect; calyx glandular-pubescent; corolla yellow, 1.5-2.5 cm. long; anthers included. Fleshy perennial herb to 0.5 m. Coastal waste areas. South peninsula (Lee, Monroe counties); rare. Winter. [*Kalanchoe integra* (Medic.) Kuntze var. *crenata* (Andr.) Cuf.; *Kalanchoe laciniata*, misapplied]

* ***Kalanchoe crenata*** (Andr.) Haw.

3. Leaves crowded at base of plant, with brown-edged crenations on upper half; flowers pendent; calyx glabrous; corolla dull red to orange, 2.0-2.5 cm. long; anthers protruding. Fleshy perennial herb to 0.4 m. Coastal waste areas. South peninsula (Lee, Martin counties); rare. Winter.

* ***Kalanchoe fedtschenkoi*** Hamet & Perr.

2. Plants unbranched (monocarpic), growing for a few years, flowering only once, then dying; leaves narrowly cylindrical or broadly to narrowly deltoid, untoothed or coarsely and sharply serrate, with prominent plantlet-bearing flange at each sinus; flowers pendent.

4. Leaves whorled, mostly in 3's (or alternate on upper stem), subcylindric (grooved on upper surface), sessile, toothed only at apex, 3-5 cm. long; corolla salmon, 2.5-3.0 cm. long. Monocarpic fleshy short-lived perennial herb to 2 m. Dry shelly waste areas. Coastal areas of peninsula (n. to Levy, Brevard counties); infrequent. Fall-winter. [*Kalanchoe tubiflora* (Harvey) Hamet; *Kalanchoe verticillata* S. Elliot; *Bryophyllum delagoense* (Eckl. & Zeyh.) Schinz.] CHANDELEIR-PLANT.

* ***Kalanchoe delagoensis*** Eckl. & Zeyh.

4. Leaves opposite, broad, very thick, petiolate, toothed along margin.

5. Leaf blades narrowly deltoid to broadly lanceolate; corolla dark red, 2.0-2.5 cm. long. Monocarpic fleshy short-lived perennial herb to 1.5 m. Waste areas, foundation plantings, greenhouse debris. South and central peninsula (n. to Alachua County); infrequent. Winter. A hybrid of *K.*

daigremontiana and *K. delagoensis*, reproducing exuberantly by viviparous leaf-margin plantlets.

* ***Kalanchoe houghtonii*** D. B. Ward

5. Leaf blades broadly deltoid; corolla dusky rose, 1.5-2.0 cm. long. Monocarpic fleshy short-lived perennial herb to 0.8 m. Dry waste areas. South and central peninsula (n. to Brevard County); infrequent. Winter.
DEVIL'S BACKBONE.

* ***Kalanchoe daigremontiana*** Hamet & Perr.

Excluded names:

Kalanchoe blossfeldiana Poelln.

Widely sold by home garden stores, and garden waifs sparingly seen in a South Florida hammock (Lee Co.). But apparently sterile, and not truly escaped.

Kalanchoe gastonis-bonnieri Hamet & Perr.

Reported by Spongberg (1978) to be "well established" on Sanibel Id.; and by Wunderlin (1982, 1998) as "rare" in Lee Co. The sole basis appears to be a 1972 collection (Brumbach 7837 - FLAS), said to be "escaped & well established" on Sanibel Id. However the specimen, though poor, is of *K. pinnata*, a species not recognized by the collector until 1974, when he found it abundant at the same location.

Kalanchoe laciniata (L.) DC.

Reported by Wunderlin (1998) as escaped in Lee and Monroe counties, apparently based on a 1974 Sanibel Id. collection (Brumbach 8209 - FLAS) that, though annotated *K. laciniata*, is typical *K. crenata*.

Kalanchoe laxiflora Baker

Bryophyllum crenatum Baker

Cited as "reported" for Florida by Long & Lakela (1971), but not by whom. Unknown in Florida outside of cultivation.

Kalanchoe marmorata Baker

Kalanchoe grandiflora A. Rich.

Reported as naturalized, "sometimes in extensive colonies" (Long & Lakela, 1971). No such colonies nor individual plants are known outside of cultivation. A distinctive species; a misidentification is difficult to visualize.

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[1] This paper is a continuation of a series begun in 1977. The "amplified key" format employed here is designed to present in compact form the basic morphological framework of a conventional dichotomous key, as well as data on habitat, range, and frequency. Amplified keys are being prepared for all genera of the Florida vascular flora; the present series is restricted to genera where a new combination is required or a special situation merits extended discussion.

BIOLOGICAL STATUS OF *PACKERA THURBERI* (ASTERACEAE: SENECIONEAE) AND ITS RELATIONSHIP TO *PACKERA NEOMEXICANA*

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ABSTRACT

Packera thurberi (A. Gray) B. L. Turner (= *Senecio thurberi* A. Gray) is recognized as an earlier name for *Packera tridenticulata* and the appropriate new combination is proposed. The relationship of this species with *P. neomexicana* is discussed, and the distribution of each is mapped.

KEY WORDS: *Packera*, *Senecio*, Asteraceae

Turner (1993) discussed the taxonomy and nomenclature of *Senecio neomexicanus* A. Gray. In this, he noted that the taxon was typified by a Lemmon collection from the Santa Catalina Mountains, Pima Co., Arizona. He commented further that it

> Was first proposed in 1883 without description. Gray, nevertheless, noted that the name was "given to a troublesome species, collected in New Mexico by *Wright*, *Thurber*, *Henry*, *Greene*, etc., in Arizona recently by *Lemmon* and *Pringle*, and within the borders of California by *Parish*, specimens of which have been variously and dubiously referred to *S. fendleri*, *multilobus*, *aureus*, etc." Gray subsequently [in 1884] formally described the species, noting in his protologue that it occurred in "Mountains and wooded hills of New Mexico..."

Trock (1999) in her doctoral dissertation of *Packera* (a segregate of *Senecio* s.l.) was apparently unaware of, or neglected to cite, the above publication. In her assessment of *Senecio neomexicanus*

she largely followed the work of Barkley (1978), who recognized four varieties in the complex. The typical var. *neomexicanus* was said to be typified by a "holotype" at GH collected by C. Wright in the Organ Mountains of New Mexico. But, as noted above, several collections were mentioned in the protologue of *S. neomexicanus*. I was obliged therefore to select a lectotype from among them, this not mentioned by Trock in her publication but accounted for by Freeman and Barkley (1995) in their treatment of *Packera* for Mexico.

Trock (1999), following Barkley, placed *Senecio thurberi* in synonymy under her concept of *Packera neomexicana*. Trock (2006), and Barkley himself, while listing *S. thurberi* as a synonym of *S. neomexicana*, noted the holotype (GH!) to be "an abnormal, narrow-leaved collection whose disposition here is purely provisional." Trock also noted that if the "type of *Senecio thurberi* A. Gray belongs within the subscription of *Packera neomexicana* (see T.M Barkley 1978), a new combination in *Packera* will be necessary."

Indeed, in my examination of type material of *S. thurberi*, I concluded that the elements concerned belonged to an alliance of taxa (listed in the above publications) centering about *Packera tridenticulata*, as treated by both Barkley and Trock. So conceived, *Senecio thurberi* becomes the earliest available name for the complex, and I have little hesitation in making the following combination to accommodate this conclusion:

Packera thurberi (A. Gray) B.L. Turner, **comb. nov.**
Based upon *Senecio thurberi* A. Gray, Proc. Amer. Acad. Nat. Sci. Philadelphia 68. 1863.
TYPE: NEW MEXICO. Grant Co.: Hillsides, Copper Mines, May 1852, *Thurber 210* (Lectotype: GH!; isolectotype TEX!).

Trock (2003), in her discussion of the taxa of *Packera* in Colorado, distinguished *P. neomexicana* (var. *mutabilis*) from *P. thurberi* (as *P. tridenticulata*) by vestiture: tomentose in the former; glabrous in the latter. But, she notes in considerable detail the complexities of the taxa, drawing upon "Four characters, when taken in combination," to help to distinguish the two taxa. Those listed were:

1.) "geography," *tridenticulata* more eastern, but has "jumped" upon occasion the "Rocky Mountain Front."
2.) "clumps," *tridenticulata* reportedly having more clumps (4+) from a stout tap root, *neomexicana* with fewer (2-3).
3.) achene size, *tridenticulata* with smaller ("1.5-2.5 mm long"); *neomexicana* with larger (1.5-2.5 mm long, as given in her description).
4.) pubescence, mostly glabrous in *tridenticulata*, but this condition grading here and there to into the more western, more pubescent *neomexicana*.

In short, Trock, for the state of Colorado, presents a tortuous account of how these two taxa differ, to say nothing of the variation that might occur elsewhere, hence my reduction or inclusion of the Colorado taxa of *P. neomexicana* as envisioned by Trock (2003) within the framework of a widespread highly variable *Packera thurberi* (including the several varieties of *P. neomexicana* recognized by yet other workers, and those of Trock 2006).

Admittedly, all of this is a sort of Pandora's Box, but my many years of field work in the areas concerned has led me to believe that there is biological reality in the recognition of a more northern, highly variable, *P. thurberi* (including *P. tridenticulata*), and a more southern, less widespread, less variable, *P. neomexicana.* The distribution of the latter is shown in greater detail in Barkley's treatment of these taxa (as *P. neomexicana* var. *neomexicana*, his Fig. 3), this largely reproduced in my Fig. 1 of the present article. My map showing the distribution of *P. thurberi* (Fig. 2) is based upon Barkley's concept of *P. neomexicana* var. *mutabilis*, along with the distributional data of *P. tridenticulata,* as envisioned by Trock (1999, 2003, 2006).

ACKNOWLEDGEMENTS

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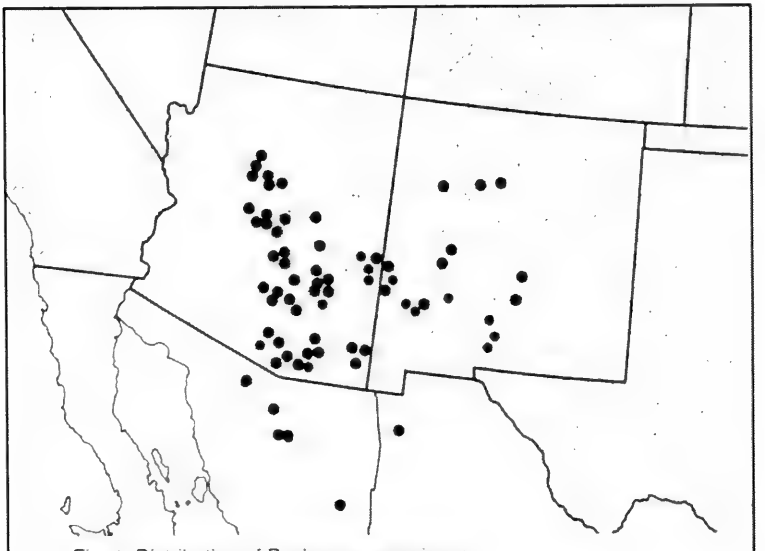

Fig. 1. Distribution of Packera neomexicana.

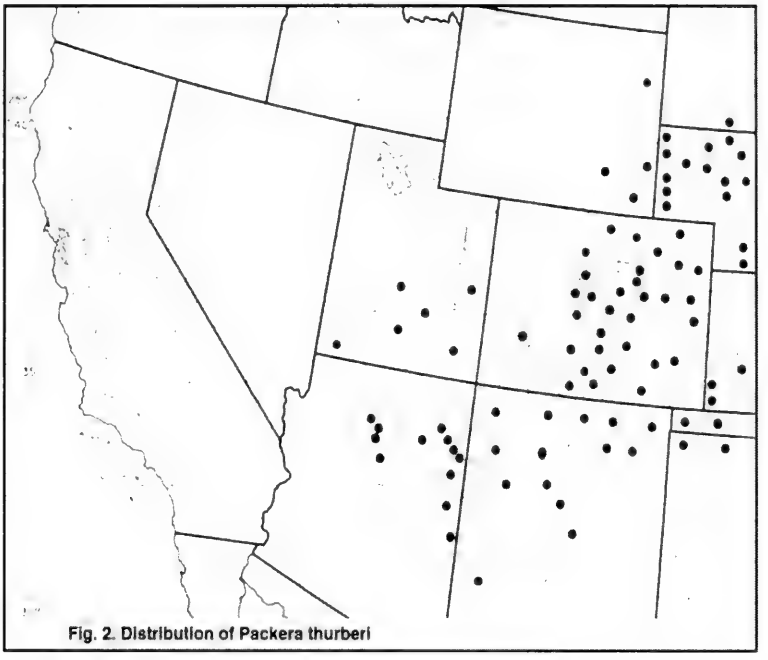

Fig. 2. Distribution of Packera thurberi

OVERVIEW OF THE SECTION PLATYPTERIS OF *VERBESINA* (ASTERACEAE) AND DESCRIPTION OF A NEW SPECIES

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ABSTRACT

A systematic overview of the sect. Platypteris of *Verbesina* is provided, including eight species. One of these, *V. jimrobbinsii*, is newly described from the fabric of McVaugh's concept of *V. platypteris*. A key to the species is provided, along with maps showing their distributions.

KEY WORDS: *Verbesina*, Asteraceae, Heliantheae, *Platypteris,* Mexico, Jalisco, Oaxaca.

Olsen (1988) provided a revision of the section Platypteris (Kunth) DC. of *Verbesina*. In this he recognized five species: *V. crocata* (the generitype), *V. fraseri, V. lottiana*, *V. ovatifolia*, and *V. vallartana*. Subsequently, an additional species, *V. barrancae*, was added to the complex by Harker and Jimenez (2002). The present additions of *V. platyptera* and *V. jimrobbinsii* bring to eight, the number of specific taxa recognized as belonging to the section.

The section Platypteris was first established as a genus by Kunth but subsequently treated as a section of *Verbesina* by DeCandolle, this arrangement followed by Robinson and Greenman (1899) in their synopsis of *Verbesina*.

Species of the section Platypteris, as understood by previous workers, including Olsen (1988), are recognized by their shrubby habits, large eradiate heads, and markedly winged stems. Interestingly, at the time of Olsen's treatment an additional taxon lurked as a possible

member of the section, this being *V. platyptera* Schultz-Bip. in Klatt. Indeed, Olsen, in a letter addressed to me in June of 1987 stated that "I am still undecided about whether I should go ahead and sink *V. platyptera* into the Section." Robinson and Greenman (1899) treated *V. platyptera* as the sole member of their section Stenocarpha, which was largely distinguished from section Platypteris by its smaller heads, ray florets, and sinuate-pinnatifid leaves.

Verbesina platyptera, in my opinion, should be positioned in the section Platypteris, as suggested by Olsen. It has all of the characters of that taxon except that it possesses ray florets, and the outer involucral bracts are somewhat foliaceous and reflex at maturity. Panero and Jansen (1997) studied the chloroplast DNA of several species of the section Platypteris, all of these hanging close one to the other. Unfortunately, they did not sample *V. platyptera* and/or *V. jimrobbinsii*, but I have little doubt but what such data will vouchsafe their position in section Platypteris.

The type of *V. platyptera* was obtained from along the Pacific shore of Oaxaca (Puerto de Santa Cruz, *Liebmann 454*). Nevertheless, McVaugh (1984), in his treatment of *Verbesina* for Flora Novo-Galeciana applied the specific name to inland, more montane, populations of what I take to be a very different taxon, described herein as *V. jimrobbinsii*. Olsen (1988) provided a key to the taxa of section Platypteris known to him and since, while listed below, and need no reevaluation, they are not further discussed in the present paper. Harker and Jimenez (2002) described *V. barrancae*, producing a key to the more western species in Mexico so as to accommodate their views. I have modified their key so as to accommodate my two new additions to the section, as follows:

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Key to the western species of Section Platypteris in Mexico

1. Ray florets absent .....**(3)**
1. Ray florets present, albeit small and inconspicuous.....**(2)**
   2. Leaves relatively small, merely 3-lobed, not pinnately dissected; ligules of ray florets 1-3 mm long; near Santa Cruz, Oaxaca .....**V. platyptera**

2. Leaves relatively large, pinnately divided or dissected; ligules of ray florets minute, inconspicuous; dry forests, 300-600 m in Jalisco, Michoacan, and Guerrero.....**V. jimrobbinsii**

3. Corollas of florets evenly hispid throughout.....**V. vallartana**
3. Corollas unevenly pubescent, mostly glabrous.....**(4)**

4. Heads 4-8 mm high; peduncles mostly 8-16 cm long...**V. lottiana**
4. Heads 10-25 mm high; peduncles 0.5-7.0 cm long .....**(5)**

5. Anthers uniformly orange or yellow-orange throughout; widespread .....**V. crocata**
5. Anthers dark with cream-colored appendages; environs of Guadalajara.....**V. barrancae**

VERBESINA BARRANCAE Harker & Jimenez-Reyes, Brittonia 54: 182. 2002. **Map 1**

This taxon is known only by collections from barrancas near the city of Guadalajara. It is closely related to *V. crocata*, as well-noted by its authors, who called to the fore both exomorphic and palynological features by which to distinguish between the two.

VERBESINA CROCATA (Cav.) Less., Syn. Gen. Compos. 232. 1832. **Map 2**

This is the most conspicuous, commonly encountered member of the section Platypteris. It is treated in some detail by Olsen (1988) and need not be further commented upon here.

VERBESINA FRASERI Hemsl., Biol. Cent.-Amer., Bot. 2: 187. 1881.

This species is known only from Central America, not having been collected in Mexico.

VERBESINA JIMROBBINSII B.L. Turner, **sp. nov. Fig. 1, Map 3**

Verbesinae platypterae Schultz-Bip. similes sed foliis profunde pinnatifidis (vs 3-lobatis vix pinnatifidis), bracteis involucri externis non foliosis, et ligulis flosculorum radii carentibus (vs ligulis praesentibus).

Arborescent shrubs 1-3 m high. **Primary stems** markedly winged, the wings 1-2 cm wide (less so on secondary stems). **Leaves** 10-30 cm long, 5-20 cm wide, deeply dissected; petioles mostly 3-6 cm long, winged throughout. **Capitulescence** a very large divaricately branched panicle, up to 20 cm long, 30 cm wide. **Heads** ca 10 mm high, 6-10 mm wide, the outer-most involucral bracts linear to linear-lanceolate, reflexed at maturity, about as long or somewhat shorter than the inner bracts. **Ray florets** 13-21, pistillate, fertile; corollas tubular 4-6 mm long, their rays poorly developed, if at all. **Disk florets** 50-60; corollas yellow, 5-7 mm long. **Achenes** as illustrated in McVaugh (1984).

TYPE: **MEXICO: JALISCO. Mpio. Cihuatlan**, "Hills between Bahia Navidad, and La Manzinilla on Bahia Tenacatita; east-facing summits 3 miles west of the Autlan-Navidad highway, in tropical forest; 550 m, 12 Nov. 1960, *Rogers McVaugh 21008* (holotype: LL; isotype LL)..

ADDITIONAL SPECIMENS EXAMINED: **MEXICO. JALISCO: Mpio. Cihuatlan,** along highway 200, 4.8 km W of intersection with highway 80, base of east-facing slopes in a seemingly tropical forest, 5 Oct 2007, *B.L. Turner et al. 7-31* [with J. Robbins, P. Waller & M. Turner] (TEX, MEXU). **MICHOACAN: Mpio. Villa Victoria,** Huizontla, "Barranca forest; shrub 3 m high," 640 m, 15 Nov 1938, *Hinton et al. 12580* (LL).

The type labels note the species to be a shrub 1-2 m high, and locally "abundant". At the type locality (exactly 4.8 km from the intersection concerned), some 40 years later, *Turner et al. 7-31* (see above) could locate only 3 plants at the site concerned, all of these at the very base of the east-facing slope along hiway 200. Certainly the taxon is not now "abundant," as noted by McVaugh, but perhaps the

species was originally collected along the top of the Sierra, as suggested by his label data. We found the plants to vary from perennial herbs (1 m high, Fig. 2), to a shrub ca. 3 m high (Fig. 3, see rear cover), the latter possessing a woody stem ca 2.5 cm in diameter.

McVaugh (1984) included this taxon in his concept of *V. platyptera*, the type from relatively low elevations (30-60 m) about the port of Santa Cruz, Oaxaca. Indeed, nearly all of his description of the latter, including its illustration, relates to what is here described as *V. jimrobbinsii*. As indicated in the above diagnosis, *V. jimrobbinsii* is readily recognized by a number of characters and is largely restricted to wetter, more montane (300-700 m) sites.

The species is named for James (Jim) Robbins, former "son-in-law" and long time live-in mate with my son Matt Turner, both of whom participated in the recollection of the taxon at its type locality in the fall of 2007. Jim also provided travel funds for the botanical venture, participating in the rediscovery of plants from the type locality.

VERBESINA LOTTIANA B.L. Turner & J. Olsen, Sida 13: 41. 1988. **Map 1**

This very distinct, markedly endemic species is known by only a few collections from the Mpio. La Huerta, along the Pacific slopes.

VERBESINA OVATIFOLIA A. Gray, Amer. Acad. Arts 19: 15. 1883. **Map 4**

This is a widespread, quite variable taxon. In Mexico, it is largely restricted to the more eastern provinces but extends eastward through Chiapas into Central America.

VERBESINA PLATYPTERA Sch. Bip. in Klatt, Leopoldina 23: 144. 1887. **Map 1**

This species is typified by material gathered from the environs of "Puerto Sta. Cruz" in southeastern Oaxaca, Mexico in 1842 by Liebmann. McVaugh (1984) confounded its ecomorphological boundaries by including in this material herein segregated as *V.*

jimrobbinsii as shown in Map 3. The latter is a species of more montane habitats (dry forests at elevations of 300-600 m); *V. platypteris* occurs at much lower elevations in deciduous forests along the Pacific coast.

Since *V. platypteris* is poorly described in the earlier literature, I provide the following, more up-to-date, description, this largely compiled from several recent collections from SERBO (Sociedad Estudio Recursos Bioticos Oaxaca), as follows: **OAXACA**: Mpio Santa Maria Huatulco, 40-55 m., *Saynes-V. 5123, 5153*, and *Martinez 373* (all at TEX).

Shrubs 2.0-2.5 m high. **Stems** densely short-pubescent with stiff spreading hairs, the vestiture up to 0.5 mm high; upper mid-stems with relatively narrow wings, 1-5 mm wide. **Leaves** mostly 3-lobed, 12-20 cm long, 8-13 cm wide, moderately pubescent on both surfaces with short, stiff, broad-based hairs; petioles 4-7 cm long, winged throughout. **Capitulescence** a terminal cyme of 3-7 heads, 6-10 cm high, 8-10 cm wide, the ultimate peduncles mostly 1-3 cm long, densely pubescent with mostly upswept hairs. **Heads** (the reflexed outer-most bracts excluded) 1.0-1.4 cm high, ca 2 cm across; involucral bracts, the outer-most, loose, leaf-like, reflexed at maturity, mostly oblanceolate, longer than the inner bracts, 10-14 mm long, 2-3 mm wide. **Receptacle** somewhat convex, 3-4 mm across; pales linear-lanceolate, 7-8 mm long. **Ray florets** ca 18, yellow; tubes 2-3 mm long, enlarged upwards and forming distinct ovate to linear ligules 1-2 mm long. **Disk florets** 60-80; corollas yellow, ca 6 mm long, more or less glabrous, except for the hispid lobes.

VERBESINA VALLARTINA B.L. Turner & J. Olsen, Sida 13: 39. 1988. **Map 3**

This taxon is known only from the states of Colima and Jalisco, reportedly occurring in tropical deciduous forests along coastal areas from 60-400 m.

ACKNOWLEDGEMENTS

I am grateful to my colleague, Guy Nesom, for the Latin diagnosis and reviewing the paper. Mr. James Robbins funded the present author's trip, for which I am most grateful, noting that he now has a better appreciation of what plant systematics in the field is about: lots of climbing, mosquitoes, spiders, and dangerous snakes lurking about one's ankles. Something new for an IBM executive!

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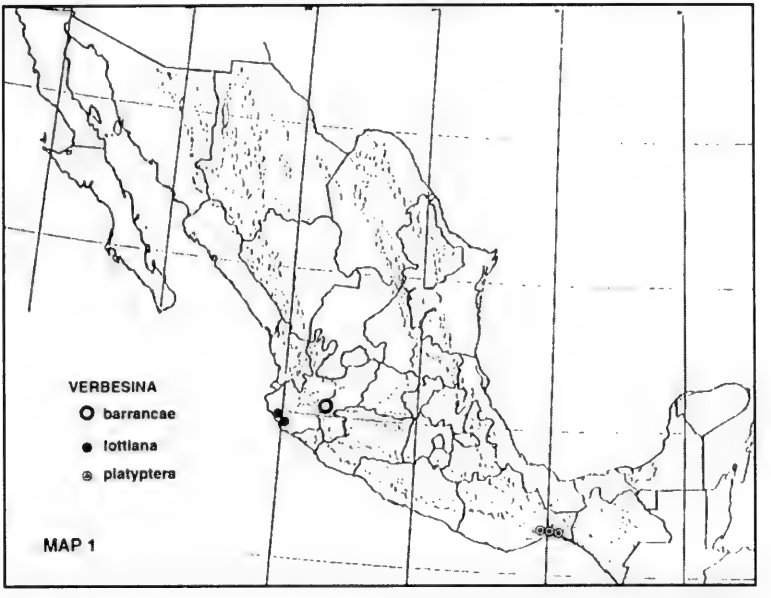
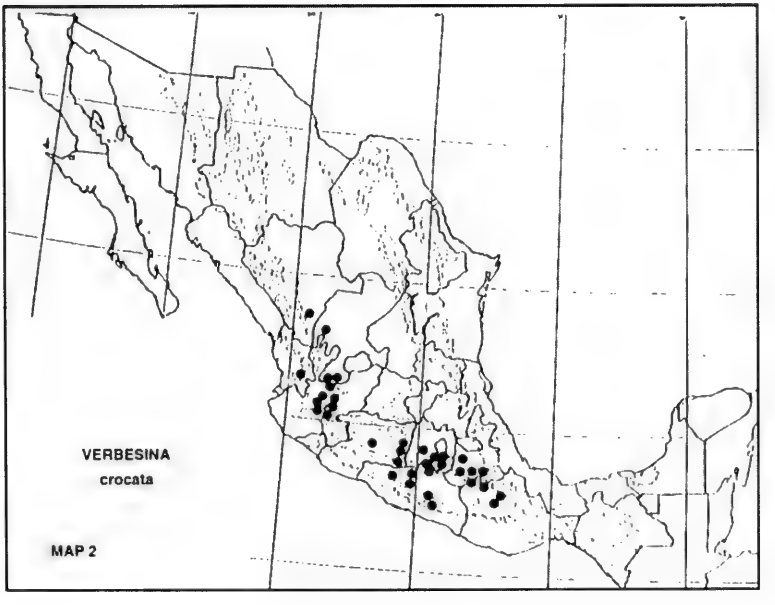
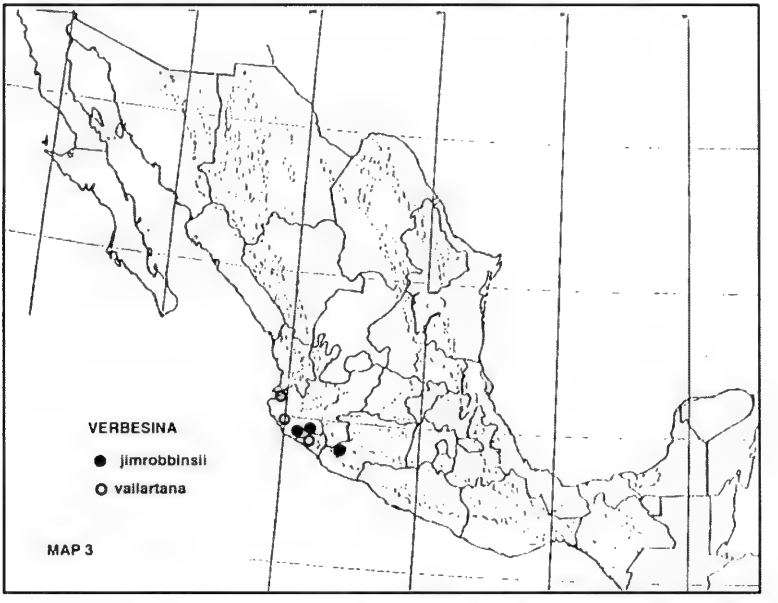
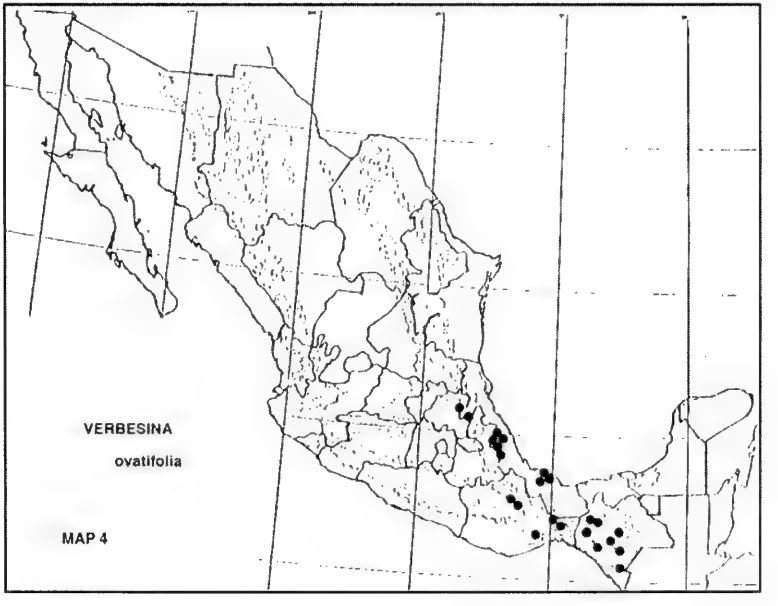
Fig. 1. *Verbesina jimrobbinsii* (Holotype: TEX).

Fig. 2. *Verbesina jimrobbinsii*, plant and flower, held next to the eponymous Jim Robbins, at type locality.


VERBESINA
barrancae
lottiana
platyptera
MAP 1


VERBESINA
crocata
MAP 2


VERBESINA
jimrobbinsii
vallartana
MAP 3


VERBESINA
ovatifolia
MAP 4

NOMENCLATURAL CHANGES AND SELECTED LECTOTYPIFICATIONS IN *CASTILLEJA* (OROBANCHACEAE)

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ABSTRACT

The following nomenclatural novelties are established in the genus *Castilleja*: ***C. affinis*** var. ***neglecta*** (Zeile) J.M. Egger, ***C. ambigua*** var. ***humboldtiensis*** (D.D. Keck) J.M. Egger, ***C. ambigua*** var. ***insalutata*** (Jeps.) J.M. Egger, ***C. bryantii*** var. ***socorrensis*** (Moran) J.M. Egger, ***C. campestris*** var. ***succulenta*** (Hoover) J.M. Egger, ***C. ctenodonta*** var. ***altorum*** (Standl. & Steyerm.) J.M. Egger, ***C. densiflora*** var. ***gracilis*** (Benth.) J.M. Egger, ***C. densiflora*** var. ***obispoënsis*** (D.D. Keck) J.M. Egger, ***C. exserta*** var. ***latifolia*** (S. Watson) J.M. Egger, ***C. exserta*** var. ***venusta*** (A. Heller) J.M. Egger, ***C. integrifolia*** var. ***chiapensis*** (Brandegee) J.M. Egger, ***C. integrifolia*** var. ***longibracteata*** (M. Martens & Galeotti) J.M. Egger, ***C. miniata*** var. ***fulva*** (Pennell) J.M. Egger, ***C. minor*** var. ***exilis*** (A. Nelson) J.M. Egger, ***C. minor*** var. ***stenantha*** (A. Gray) J.M. Egger, ***C. minor*** var. ***spiralis*** (Jeps.) J.M. Egger, ***C. moranensis*** var. ***cinerascens*** (Eastw.) J.M. Egger, ***C. pallida*** var. ***hyparctica*** (Rebrist.) J.M. Egger, ***C. pallida*** var. ***lapponica*** (Rebrist.) J.M. Egger, ***C. pallida*** var. ***saccata*** (Pennell) J. M. Egger, ***C. pallida*** var. ***yukonis*** (Pennell) J.M. Egger, ***C. pectinata*** var. ***purpusii*** (Brandegee) J.M. Egger, ***C. rubicundula*** var. ***lithospermoides*** (Benth.) J.M. Egger, ***C. subinclusa*** var. ***jepsonii*** (Bacig. & Heckard) J.M. Egger, and ***C. tenuiflora*** var. ***tancitaroana*** (G.L. Nesom) J.M. Egger. Lectotypifications are proposed for the following names: *C. longibracteata* M. Martens & Galeotti and *C. spiralis* Jeps. Complete synonymies are provided for all treated taxa.

KEY WORDS: *Castilleja*, Lectotypification, nomenclature, Orobanchaceae, *Orthocarpus*, Scrophulariaceae.

This paper proposes a number of nomenclatural changes, along with relevant lectotypifications and synonymies within the genus *Castilleja* Mutis ex L.f., formerly placed in Scrophulariaceae but now included within Orobanchaceae (Olmstead et al. 2001). Some of the *Castilleja* taxa treated here were previously placed in the genus *Orthocarpus* but were later transferred to *Castilleja* (Chuang and Heckard 1991). These changes are proposed in preparation for the upcoming treatment of *Castilleja* in the Flora of North America series and in other forthcoming regional treatments of the genus.

The nomenclatural novelties are proposed in an effort to standardize the usage of infraspecific taxa within *Castilleja* with the rank of variety, following the suggestions of Holmgren (1994), Turner and Nesom (2000) and others, and to provide a consistent nomenclatural basis for my own on-going work in the genus. Historically, such taxa in *Castilleja* have almost always been treated as either varieties or subspecies, and in only one work were both ranks employed for classification, strictly within a single species (Boivin 1952). There is no indication in the major treatments of *Castilleja* in the botanical literature that those authors treated varieties and subspecies as other than essentially of equivalent biological/evolutionary standing.

Roughly two-thirds of the major historic treatments and revisions of taxa now included in *Castilleja* in which infraspecific taxa have been assigned utilized only the rank of variety. These include the works of G. Bentham (e.g. 1846), H. A. Weddell (1857), A. Gray (e.g. 1880), A. Eastwood (e.g. 1909), D. D. Keck (e.g. 1927), M. Ownbey (e.g. 1959), N. H. Holmgren (e.g. 1984a), and G. L. Nesom (e.g. 1992). Weddell (1857) was unique in also employing the rank of subvariety in *Castilleja*. Major treatments employing the rank of subspecies include those of F. W. Pennell (e.g. 1934) and T. I. Chuang and L. R. Heckard (e.g. 1991, 1992). P. A. Munz applied subspecies in some publications (e.g. 1958), while using varieties in others (e.g. 1932). With the exception of Pennell's work, most treatments using subspecies have applied primarily to the California flora.

Among the authors of significant taxonomic treatments of *Castilleja*, only Holmgren (1971, p.26) provided an explicit description of the species concept employed in his classification, "based primarily

on morphological distinctiveness among natural populations" wherein, in most cases, "morphological continuity is correlated with geographic discontinuity." Further, "The populations within a species are all closely related and have been exchanging genes at least in recent times." I follow Holmgren's work, both in terms of species concept and in his use of variety as the basis for infraspecific groupings in this genus. Through 24 years of intensive field work involving all North American taxa and most of those in Latin America, as well as herbarium study of approximately 22,000 sheets, including almost all know type collections, my studies in the genus have, with only a few minor exceptions, confirmed Holmgren's revisionary treatments of *Castilleja* across a number of regional floras (Holmgren 1970, 1973, 1978, 1984a, 1984b,) and species groups (Holmgren 1971, 1976).

My own application of the rank of variety as the fundamental unit of infraspecific classification in *Castilleja* is essentially both functional, in defining patterns of natural variation in a consistent and meaningful manner, and biological, in reflecting and defining evolutionary trends in natural populations and establishing hypotheses upon which to base future phylogenetic investigation. I define varieties as diverging systems of natural populations within a presumed progenitor species and characterized by relatively minor but reasonably consistent patterns of morphological divergence, combined with varying degrees and combinations of ecological and/or geographical partitioning. While some overlap in the defining characteristics is allowed, as one would expect in infraspecific groupings, any such overlaps should be limited in extent and non-clinal, and the taxa involved should still be generally separable through the examination of suites of characters.

The following new combinations and changes of status are here established. Full synonymies and locations of verified type specimens are provided for each name, and relevant lectotypifications are proposed where needed. Type collection citations are extracted from the protologues and amended where possible with additional documentation from the original herbarium labels. Any such label information is enclosed in parentheses. Additional technical notes regarding the type collections gleaned from my own research are enclosed in brackets. I have included exclamation points following the abbreviations of institutions housing type collections of *Castilleja* I

have seen and verified. Other locations without exclamation points are from published sources and need verification, though some have been certified in recent decades by competent taxonomists. Institutional abbreviations followed by a question mark represent those listed in the protologues or in secondary sources as locations at which type material was deposited but which could not be relocated during the research for this study. Abbreviations followed by a question mark enclosed in parentheses are hypotheses as to where type material may be located for those taxa for which neither a location was listed in the protologue nor any specimens found during this study.

Though not so arranged in the treatments below, the proposed nomenclatural changes can be divided into three categories. The first category, consisting of 12 novelties, provides varietal status to taxa previously treated as subspecies within the same species in order to standardize nomenclature in *Castilleja*. My conception of the identities and circumscriptions of the taxa included in this first group are essentially identical to that of the authors of the subspecies names, so little further comment is necessary. The second category, consisting of 12 novelties, includes names previously used only at the species level or as subspecies of different taxa but which my research indicates are better treated as varieties of the names indicated below. The third category includes a single new combination, reassigning a named variety of a parent taxon that is now known to be synonymous with an earlier-published name. Further explanatory comments are provided below for each taxon in the second and third groups of new combinations. Finally, I propose two lectotypifications necessary to provide an unequivocal basis for the identity of the taxa treated in this paper. Additional lectotypifications are needed in *Castilleja* and will be addressed in future publications. The accepted taxa are presented in alphabetical order, and their arrangement is of no taxonomic significance. Keys to all of the treated taxa and their relatives will be presented in later publications, including the Flora of North America and the Flora Mesoamericana.

Castilleja affinis Hook. & Arn. var. ***neglecta*** (Zeile) J.M. Egger, **comb. et stat. nov.** BASIONYM: *Castilleja neglecta* Zeile in W. L. Jepson, Man. Fl. Pl. Calif.: 936, 1925. *Castilleja affinis* subsp. *neglecta* (Zeile) T.I. Chuang & Heckard, Novon 2: 185, 1992.

TYPE: United States. California: Marin Co., hillsides, Tiburon, (7 Jul 1907), *Brandegee s.n.* (holotype: JEPS!; isotype: CAS!). [Note: while the collection of *Brandegee s.n.* at CAS is annotated as the "type collection" by A. Eastwood, it is unclear if this sheet contains valid type material. While it was collected in the same location as the holotype, the date on Brandegee's label for the CAS sheet is "May 1909." Unless this date is incorrect, then the CAS sheet is merely a topotype.]

Castilleja ambigua (Hook. & Arn.) T.I. Chuang & Heckard var. ***humboldtiensis*** (D.D. Keck) J.M. Egger, **comb. nov.** BASIONYM: *Orthocarpus castillejoides* Benth. var. *humboldtiensis* D.D. Keck, Proc. Calif. Acad. Sci., Ser. 4, 16: 536, 1927. *Castilleja ambigua* subsp. *humboldtiensis* (D.D. Keck) T.I. Chuang & Heckard, Syst. Bot. 16: 655, 1991. TYPE: United States. California: Humboldt Co., Eureka, (partly dry) saline flats, 20 Jun 1925, *Munz 9890* (holotype: RSA-POM!; isotypes: DS!, GH!).

Castilleja ambigua (Hook. & Arn.) T.I. Chuang & Heckard var. ***insalutata*** (Jeps.) J.M. Egger, **comb. nov.** BASIONYM: *Orthocarpus castillejoides* Benth. var. *insalutatus* Jeps., Man. Fl. Plants of Calif.: 944, 1925. *Castilleja ambigua* subsp. *insalutata* (Jeps.) T.I. Chuang & Heckard, Syst. Bot. 16: 656, 1991. TYPE: United States. California: Monterey Co., Pacific Grove, 8 Aug 1896, *Jepson 21110* (holotype: JEPS!).

Castilleja bryantii Brandegee var. ***socorrensis*** (Moran) J.M. Egger, **comb. et stat. nov.** BASIONYM: *Castilleja soccorensis* Moran, Mem. San Diego Nat. Hist. Soc. 16: 52, 1989. TYPE: México. Baja California: Isla Socorro, fairly common in low vegetation, east slope of Cerro Evermann at 980 m, near 18°46.5'N, 110°57.5'W, 4 Apr 1981, *Moran 29505* (holotype: SD!; isotypes: ARIZ!, BISH!, CAS!, ENCB, F!, MEXU, MICH!, MO!, NY!, RSA!, TEX!, UC!, US!).

Comments: Moran distinguished *C. soccorensis* from *C. bryantii* on the basis of overall stem height, calyx length, and corolla measurements. Of these, only the corolla lengths appear to be non-overlapping. While the corolla size differences do appear to be

consistent, such limited variation in a single character in *Castilleja* typically distinguishes varieties rather than full species.

Castilleja campestris (Benth.) T.I. Chuang & Heckard var. ***succulenta*** (Hoover) J.M. Egger**, comb. nov.** BASIONYM: *Orthocarpus campestris* Benth. var. *succulentus* Hoover, Leafl. W. Bot. 1: 228, 1936. *Orthocarpus succulentus* (Hoover) Hoover, Four Seasons 2(4): 14, 1968. *Castilleja campestris* subsp. *succulenta* (Hoover) T.I. Chuang & Heckard, Syst. Bot. 16: 656, 1991. TYPE: United States. California: Merced Co., Ryer, 1 May 1936, *Hoover 1076* (holotype: CAS!; isotypes: GH!, JEPS[2]!, PH[2]!, UC!, US!).

Castilleja ctenodonta Eastw. var. ***altorum*** (Standl. & Steyerm.) J. M. Egger, **comb. et stat. nov.** BASIONYM: *Castilleja altorum* Standl. & Steyerm., Field Mus. Publ. Bot. 23: 85, 1943. TYPE: Guatemala. Huehuetenango: Open alpine meadows, top of Cerro Chemalito, Sierra de los Cuchumatanes, 3.5 miles west of Santa Eulalia, alt. 3,100-3,150 meters, 2 Aug 1942, *Steyermark 49908* (holotype: F!).

Comments: In describing *C. altorum*, its authors did not distinguish it from the earlier *C. ctenodonta* and were probably unaware of the close similarity of the Guatemalan material to that of Eastwood's species, an apparently very rare taxon known only from the type collection and that of a companion of Pringle at the time he made the type collection (*Smith 539*, NY[2]!, UC!). I visited the vicinity of the type locality in August 2001 but was unable to find any plants of this distinctive species. The Guatemalan form is distinguished primarily by its generally wider leaves with less deeply denticulate margins. While the two apparently disjunct populations may eventually prove to be synonymous, I prefer to maintain them as separate varieties pending additional fieldwork.

Castilleja densiflora (Benth.) T.I. Chuang & Heckard var. ***gracilis*** (Benth.) J.M. Egger, **comb. et stat. nov.** BASIONYM: *Orthocarpus gracilis* Benth., Scroph. Ind.: 12, 1835. *Orthocarpus densiflorus* Benth. var. *gracilis* (Benth.) D.D. Keck, Proc. Calif. Acad. Sci., Ser. 4, 16: 538, 1927. *Castilleja densiflora* subsp. *gracilis* (Benth.) T.I. Chuang & Heckard, Syst. Bot. 16: 656, 1991. TYPE: United States. (Nova) California: [central coastal region,

probably in southern Monterey Co.], (1833) [1831, 1832 or possibly Nov 1833], *Douglas s.n.* (holotype: K-BENTH!; isotypes: BM!, CGE!, GH!, NY!). [Note: the Kew Herbarium labels on some of the type sheets indicate the collection date as 1833, but this is probably incorrect, as Douglas was only in California from 4-28 November of that year, during which time it is very unlikely that this Spring annual would be in full bloom. It is also possible that 1833 indicates when Bentham actually received the specimens at Kew. The isotype sheet at BM contains two stems of the type collection on the upper portion, with two unrelated collections elsewhere. According to Keck (1951), the type collection was obtained "doubtless (in the) Santa Lucia Mountains."]

Orthocarpus parishii A. Gray, Proc. Amer. Acad. Arts 17: 229, 1882. TYPE: United States. California: San Diego Co., San Jacinto Mountain, Jul 1880, *Parish & Parish 482* (holotype: GH!). [Note: some confusion exists in the literature with regard to the type collection. While A. Gray's protologue matches the collection label on the holotype sheet, Parish (1901) corrected this, stating that "The type station of this species, erroneously given as 'San Jacinto Mts.', should be: Meadows near Stonewall Mine, in the Cuyamaca Mts." Parish's correction is further supported by the fact that the San Jacinto Mountains are in Riverside Co., while the Cuyamaca Mountains are in San Diego Co., the latter county matching that given in the protologue. Compounding the confusion in the literature is reference to an apparent isotype sheet at PH(!). This sheet, incorrectly annotated as belonging to the type collection, is actually from a different collection. Its label reads: "San Jacinto Mts., Aug 1880, *Parish & Parish 408*." Confusion between this latter collection and the true type may well be the source of the original and erroneous labeling of *Parish & Parish 482*.]

Castilleja densiflora (Benth.) T.I. Chuang & Heckard var. ***obispoënsis*** (D.D. Keck) J.M. Egger, **comb. nov.** BASIONYM: *Orthocarpus densiflorus* Benth. var. *obispoënsis* D.D. Keck, Proc. Calif. Acad. Sci., Ser. 4, 16: 539, 1927. *Castilleja densiflora* subsp. *obispoënsis* (D.D. Keck) T.I. Chuang & Heckard, Syst. Bot. 16: 656, 1991. TYPE: United States. California: San Luis Obispo Co., 1 mi N of Morro, (grassy slope in view of the ocean, alt. 150 ft.), 8 Apr 1926, *Munz & Keck 10242* (holotype: RSA-POM!; isotypes: GH!, RM!).

Castilleja exserta (A. Heller) T.I. Chuang & Heckard var. ***latifolia*** (S. Watson) J.M. Egger, **comb. nov.** BASIONYM: *Orthocarpus purpurascens* Benth. var. *latifolius* S. Watson, Bot. King's Exped.: 458, 1871. *Castilleja exserta* subsp. *latifolia* (S. Watson) T.I. Chuang & Heckard, Syst. Bot. 16: 657, 1991. TYPE: United States. California: Mendocino Co., seacoast at Noyo, *Bolander 6538* (holotype: GH!; isotypes: UC[2]!).

Orthocarpus purpurascens Benth. var. *multicaulis* Jeps., Man. Fl. Plants of Calif.: 944, 1925. TYPE: United States. California: Mendocino Co., Ft. Bragg, 1914, *Mathews s.n.* (holotype: JEPS!).

Castilleja exserta (A. Heller) T.I. Chuang & Heckard var. ***venusta*** (A. Heller) J.M. Egger, **comb. nov.** BASIONYM: *Orthocarpus venustus* A. Heller, Muhlenbergia 2: 141, 1906. *Orthocarpus purpurascens* Benth. var. *venustus* (A. Heller) D.D. Keck. Proc. Calif. Acad. Sci., Ser. 4, 16: 542, 1927. *Castilleja exserta* subsp. *venusta* (A. Heller) T.I. Chuang & Heckard, Syst. Bot. 16: 657, 1991. TYPE: United States. California: San Bernardino Co., Kramer, in the Mojave Desert in sand, 13 Apr 1905, *A. A. Heller 7677* (holotype: BKL!; isotypes: BM!, CAS!, F!, GH!, K!, MO, MSC!, NY!, PH!, UC!, US!).

Orthocarpus purpurascens Benth. var. *ornatus* Jeps., Man. Fl. Plants of Calif.: 944, 1925. *Orthocarpus ornatus* A. Heller ex Jeps., Man. Fl. Plants of Calif.: 944, 1925, nomen subnudum. TYPE: United States. California: San Bernardino Co., (sandy wash), Calico Wash, Barstow, central Mojave Desert, (2,500 ft.), 28 Apr 1914, *Jepson 5819* (holotype: JEPS!). [Note: as far as I can determine, Heller never published this name directly.]

Castilleja integrifolia L.f. var. ***chiapensis*** (Brandegee) J.M. Egger, **comb. et stat. nov.** BASIONYM: *Castilleja chiapensis* Brandegee, Univ. Calif. Publ. Bot. 6: 62, 1914. TYPE: México. Chiapas: in the highest region of Cerro del Boqueron, Aug 1913, *Purpus 6884* (holotype: UC!; isotypes: F!, GH!, NY!, US!).

Comments: The widespread and complex *C. integrifolia* consists of a widely distributed nominate variety, extending from the northern Andes of South America northward into the Sierras of northeastern Mexico, and a number of distinctive, narrowly distributed endemic

forms, particularly in southwestern México and northern Central America. Some of these localized offshoots, such as the recently described *Castilleja albobarbata* H.H. Iltis & G. L. Nesom (Iltis et al. 2003), may well have diverged sufficiently to be regarded as full species, though most will require further study before unequivocal determination of rank. However, two forms are now well enough known to confirm them as valid taxa but not so fully divergent from the nominate form as to warrant full species rank. The first of these is var. *chiapensis*, based on its highly distinctive, densely villous, golden-yellow pubescence and its limited but well-defined distribution as an endemic of the Sierra Madre de Chiapas of southern Chiapas, México. Nesom (1992) mentions apical lobes or teeth on the terminal bracts as also characteristic of this form, but my studies show this to be occasional and sporadic in var. *chiapensis*, unlike the case with the following variety.

Castilleja integrifolia L.f. var. ***longibracteata*** (M. Martens & Galeotti) J.M. Egger, **comb. & stat. nov.** BASIONYM: *Castilleja longibracteata* M. Martens & Galeotti, Bull. Acad. Roy. Soc. Bruxelles 12(2): 28, 1845. **LECTOTYPE**, designated here: México. Oaxaca: dans les bois de Juquila del Sur (cote pacifique d'Oaxaca), 5000 pieds, Sep 1840, *Galeotti 988*, in part (hololectotype: BR!; isolectotypes: BR[2]!, G! K?, P!). [Note: while a second location is mentioned in the protologue ("a Talea et dans le Rincon, cordill. orientale d'Oaxaca"), all three sheets of *Galeotti 988* at BR mention only the first locality listed above, and it is clear that collection was intended by the authors to serve as the type. I could not locate any sheets bearing a label for the second location mentioned in the protologue. The isolectotype sheet at G, while bearing the type number, appears to be a specimen of *Castilleja tenuiflora* Benth., and it lists somewhat different location information, "Bois de Cote, de la Mer Pacifique, 5500 pieds." It seems likely that this specimen was incorrectly numbered and should not be regarded as authentic material from the type collection.]

Comments: Though first described in 1845, this poorly understood taxon was overlooked until recent field work revealed it to be a morphologically-consistent and fairly common endemic in the Sierras surrounding the valley of Oaxaca in southern Mexico. Its

range apparently extends from the Sierra Madre del Sur south of Miahuatlan, near which it was first collected, northward to the Sierra Juarez, north of the city of Oaxaca. The plants are unique in their distinctively broad and strongly fimbriate terminal bracts, clearly displayed in the specimens of the type collection, and unusually long, prominently colored corollas, but they are otherwise like the nominate variety. A key to some of the taxa associated with *C. integrifolia* will be presented in the forthcoming treatment of the genus in the Flora Mesoamericana.

Castilleja miniata Douglas ex Hook. var. ***fulva*** (Pennell) J.M. Egger, **comb. et stat. nov.** BASIONYM: *Castilleja fulva* Pennell, Proc. Acad. Nat. Sci. Phila. 86: 540, 1934. TYPE: Canada. British Columbia: North slopes of Peace R. Valley, high bluffs at Hudson Hope, (NW of the town, about 56°2'N, 121°55'W, alt. 2000 feet), 16 Jun 1932, *Raup & Abbe 3602* (holotype: PH!; isotypes: CAN!, F!, GH[2]!, NY!, S!, US!). [Note: some isotype sheets list the elevation as "2100 ft".]

Comments: This little-known form is fairly common only in a limited area near the type locality but extending eastward into adjacent central-western Alberta. It is distinguished by its usually yellowish to apricot-orange and densely villous inflorescence.

Castilleja minor (A. Gray) A. Gray var. ***exilis*** (A. Nelson) J.M. Egger, **comb. et stat. nov.** BASIONYM: *Castilleja exilis* A. Nelson, Proc. Biol. Soc. Wash. 17: 100, 1904. *Castilleja stricta* Rydb., Mem. N.Y. Bot. Gard. 1: 354, 1900, later homonym, not Benth., 1846. TYPE: United States. Nevada: [Elko Co.], Ruby Valley, 6000 ft., Aug 1868, *Watson 809* (holotype: NY!; isotypes: US!, YU!). [Note: Nelson's name is simply a renaming of Rydberg's later homonym.]

Euchroma lanceolata Nutt. ex A. Gray, Amer. J. Sci. Arts 84: 336, 1862, nomen subnudum. TYPE: United States. (Lewis River), [in 1834], *Nuttall s.n.* (holotype: PH!). [Note: this name was listed by Gray as a synonym of *Castilleja affinis* Hook. & Arn. var. *minor* A. Gray. The type collection is represented by a single stem mounted in the center of the same sheet as that containing the type of *Euchroma simplex* Nutt. ex A. Gray (see below). Apparently, these two specimens were collected at or near the same location, as

the labels are identical except for the names assigned to them by Nuttall.]

Euchroma simplex Nutt. ex A. Gray, Amer. J. Sci. Arts 84: 336, 1862, nomen subnudum. TYPE: United States. (Oregon) [Territory]: (Lewis River), [1834], *Nuttall s.n.* (holotype: PH!; isotype: K-HOOK!). [Note: this name was listed by A. Gray as a synonym of *Castilleja affinis* Hook. & Arn. var. *minor* A. Gray. The type collection is represented by a single stem mounted on the left side of the same sheet as that containing the type of *Euchroma lanceolata* Nutt. ex A. Gray.]

Comments: *Castilleja minor* is a complex species that has been treated at various times as either four separate species or as two subspecies distinguished by the length of the corolla beak. My conclusion after review of hundreds of specimens and scores of populations from throughout the range of the species is that this complex is best treated as a single polymorphic species consisting of four essentially parapatric varieties distinguished by relatively minor but reasonably consistent characters. A key to these varieties will be presented in the treatment of *Castilleja* in the Flora of North America.

Castilleja minor (A. Gray) A. Gray var. ***stenantha*** (A. Gray) J.M. Egger, **comb. et stat. nov.** BASIONYM: *Castilleja stenantha* A. Gray, Syn. Fl. N. Amer. 2: 295, 1878. *Castilleja stenanthe* (A. Gray) L. Abrams, Fl. Los Angeles: 369, 1904 [transcriptional error of A. Gray's original name]. TYPE: United States. California: [Monterey Co.?], moist grounds ...from Monterey to San Diego and through the southern part of the Sierra Nevada, (moist, shady places, Carmel River, 1848), *Hartweg 1897* (*134*) (= *Pl. Hartweg. 329*, "in part") (holotype: GH!; isotypes: BM!, K-BENTH!, K-HOOK!, NY!). [Note: on the holotype sheet, only the second stem from the left is material from *Hartweg 1897*. The sheet also contains two collections of the present taxon obtained by others. The isotype sheet at K-BENTH contains one stem of the type collection mounted on the right side with an unrelated collection of the left, while the sheet at K-HOOK contains one stem on the left side along with two unrelated collections elsewhere. The isotype sheet at BM contains a single stem of the type collection, mounted on the far left, along with two stems from a different collection

found elsewhere on the sheet. Pennell (1951) lists the type locality as the "vicinity of Fort Tejon", which is located in southwestern Kern Co., CA, an assertion for which there is little evidence, especially considering the fact that the holotype sheet specifies "Carmel River" as the location of Hartweg's collection. The holotype sheet is the only label to associate the added (field number?) 134 to Hartweg's collection number.]

Castilleja minor (A. Gray) A. Gray var. ***spiralis*** (Jeps.) J.M. Egger, **comb. et stat. nov.** BASIONYM: *Castilleja spiralis* Jeps., Fl. W. Mid. Calif. 412, 1901. *Castilleja stenantha* A. Gray subsp. *spiralis* (Jeps.) Munz, Aliso 4: 98, 1958. *Castilleja minor* subsp. *spiralis* (Jeps.) T.I. Chuang & Heckard, Novon 2: 188, 1992. **LECTOTYPE**, designated here: United States. California: Napa Co., moist rivulets, Butt's Canyon, (Pope Valley to Coyote Valley), northern Napa Co., 13 Jul 1897, *Jepson 21113, sheet 2 of 2* (hololectotype: JEPS!; isolectotype: JEPS!). [Note: the lectotypification is necessary because the two sheets of the type collection at JEPS have never been distinguished in the literature. The hololectotype sheet is JEPS #2513, labeled "sheet 2 of 2", and the isolectotype is JEPS #2514, labeled "sheet 1 of 2." The sheet chosen as the hololectotype bears flowering stems, while the other sheet contains only younger, non-flowering stems.]

Castilleja moranensis Kunth var. ***cinerascens*** (Eastw.) J.M. Egger, **comb. nov.** BASIONYM: *Castilleja schaffneri* Hemsl. var. *cinerascens* Eastw., Proc. Amer. Acad. Arts 44: 573, 1909. TYPE: México. Puebla: dry hills about Chalchicomula [= Ciudad Serdan], alt. 2592 m (8,500 ft.), 27 Jul 1901, *Pringle 8545* [= field number *187*] (holotype: GH!; isotypes: BKL!, CM!, ENCB!, K!, LY-BON[2]! MEXU!, MO!, MSC!, NY!, P!, PH[2]!, S!, UC!, US!, WIS[2]!). [Note: according to Pringle's diaries, the type collection was made "a little to the west of town" (of Chalchicomula).]

Comments: This long-overlooked taxon, known primarily from the ample type collection and a few smaller collections from near the type locality, was originally described as a variety of *Castilleja schaffneri* Hemsl. Recent examination of relevant types makes it clear that the latter name is synonymous with the earlier *C. moranensis* Kunth, necessitating the new combination. Apparently,

this form has not been collected in decades, and a search of the vicinity of the type locality in 2000 failed to locate any plants. Until new populations can be located, this taxon should be considered as very rare or possibly extinct.

Castilleja pallida (L.) Spreng. var. ***hyparctica*** (Rebrist.) J.M. Egger, **comb. et stat. nov.** BASIONYM: *Castilleja hyparctica* Rebrist., Nov. Syst. Pl. Vasc. 1 (Leningrad): 289, 1964. *Castilleja pallida* subsp. *hyparctica* (Rebrist.) A. Löve & D. Löve, Bot. Notiser 128: 518, 1976. TYPE: [Russia.] Ad Kolymam inferiorem, prope pagum Penteleicha, 12 Jul 1950, *Nepli s.n.* (holotype: LE).

Castilleja pallida (L.) Spreng. var. ***lapponica*** (Gand. ex Rebrist.) J.M. Egger, **comb. et stat. nov.** BASIONYM: *Castilleja lapponica* Gand. ex Rebrist., Nov. Syst. Pl. Vasc. 1 (Leningrad): 293, 1964. *Castilleja lapponica* Gand., Fl. Europ. 18: 25, 1889, nomen nudum. *Castilleja pallida* subsp. *lapponica* (Gand. ex Rebrist.) A. Löve & D. Löve, Bot. Notiser 128: 518, 1976. TYPE: Russia. Lapponia rossica. Ad Svjatoines, 12 Aug 1880, *Enwald & Knabe s.n.* (holotype: LY-GAN!; isotype: S!, TUR!). [Note: the labels on the apparent isotypes are identical to that of the holotype, except that the date on the former sheet is listed as 10 Aug 1880. While this name was originally listed by Gandoger, Rebristaya was the first to provide a proper diagnosis.]

Castilleja angustifolia Gand., Fl. Europ. 18: 25, 1889, nomen nudum and later homonym, not (Nutt.) G. Don, 1838. TYPE: Russia. Lapponia rossica. Hab. Svjatoines, 12 Aug 1880, *Enwald s.n.* (holotype: LY-GAN!). [Note: the type of *Castilleja angustifolia* Gand. was collected on the same date and by the same primary collector as the type of *Castilleja lapponica* Gand. ex Rebrist., and the two may well be different sheets of the same collection, with slightly different label information. The two appear to be no more than minor variants of the same taxon.]

Castilleja pallida (L.) Spreng. var. *borealis* Pohle ex Rebrist., Arktich. Fl. SSSR 8: 284, 1980, nomen subnudum. TYPE: not cited or located for this study (holotype: LE[?]). [Note: this name is based on an unknown herbarium sheet, presumably at LE, and is listed by O. V. Rebristaya as a synonym of *Castilleja lapponica*

Gand. *ex* Rebrist. Apparently, the present varietal name was never published by R. R. Pohle.]

Castilleja schrenkii Rebrist. Nov. Syst. Pl. Vasc. 1 (Leningrad): 293, 1964. TYPE: Russia. Lapponia rossica, 1838, *Schrenk s.n.* (holotype: LE; isotype: LY-GAN!).

Castilleja pallida (L.) Spreng. var. ***saccata*** (Pennell) J.M. Egger, **comb. et stat. nov.** BASIONYM: *Castilleja pallida* (L.) Spreng. subsp. *saccata* Pennell, Proc. Acad. Nat. Sci. Phila. 86: 523, 1934. TYPE: Russia. River banks, 20 mi. S of Ustj-Kamchatka, Kamchatka, Jul 1925, *Eyerdam 53* (holotype: PH!).

Castilleja olgae A.P. Khokhr. Biol. Rast. i Fl. Sev. Dal'n Vostok 16, 1981. TYPE: Russia. Chamchatka [= Kamchatka], in vicinity of Petropavlovsk, Tri Brata, 19 Sep 1978, *Mazurenko & Khokhrjakov s.n.* (holotype: MW!).

Castilleja pavlovii Rebrist. Nov. Syst. Pl. Vasc. 1 (Leningrad): 294, 1964. *Castilleja pallida* (L.) Spreng. subsp. *pavlovii* (Rebrist.) A. Löve & D. Löve, Bot. Notiser 128: 518, 1976. TYPE: Russia. Kamczatka [= Kamchatka], in pratis secus alveum fl. Leyvi Abgin sub monticulo Opalskaja, 27 Aug 1935, *Pavlov s.n.* (holotype: LE, isotype: MW!).

Castilleja pallida (L.) Spreng. var. ***yukonis*** (Pennell) J.M. Egger, **comb. et stat. nov.** BASIONYM: *Castilleja yukonis* Pennell, Not. Nat. Acad. Nat. Sci. Phila. 86: 531, 1934. *Castilleja tristis* W. Wight ex Standl. var. *pubens* W. Wight ex Standl. in J.B. Mertie, U.S. Dept. Inter. Geol. Surv. Bull. 810: 108, 1929, nomen nudum and superfluous. TYPE: Canada. Yukon: dry gravelly slopes and hillsides, Lewes River, (60°23'N, 134°49'W), 24 Jun 1899, *Gorman 1056* (holotype: CAN!; isotypes: NY!, PH!, S!, U, US!). [Note: the listing of the name *C. tristis* var. *pubens* in synonymy here is based on an annotation as "Type" of the sheet at US which later became an isotype of the properly published *C. yukonis*. Pennell also annotated the isotype sheet of *C. yukonis* at PH as the type collection of "*C. tristis* var. *pubens* Wight, ined." In addition, Hultén (1949) states, "In using this name Wight alludes to the plant which is here called *C. yukonis*," but he does not explain upon what evidence his statement is based. Hultén did not cite a lectotype for this name. Standley's publication of this name without

type or comment was likely intended simply to identify collections supplied to him by Mertie and was not intended by Standley as a typification. Apparently, Wight never published the name *C. tristis* var. *pubens*.]

Castilleja muelleri Pennell. Not. Nat. Acad. Nat. Sci. Phila. 86: 533, 1934. TYPE: Canada. Yukon: Lake Kluane to Donjek (Don Jek) River, 11-27 Aug 1920, *Müeller s.n.* (holotype: PH!; isotype: PH!).

Comments: The *C. pallida* complex is perhaps the most widespread and taxonomically confusing group in *Castilleja*. Extending across virtually all the Palearctic region (except for Scandinavia) and most of the western and central Nearctic, it is characterized by numerous little know and recently evolved regional and polyploid forms. Rebristaya (1964) is the only author thus far to attempt a comprehensive revision of these plants. While her treatments are extensive, detailed, and highly valuable as a preliminary assessment of the diversity present in *Castilleja* across this broad area, they lack the perspective provided by knowledge of the patterns of species diversity and variation throughout the primary range of the genus in sub-arctic North America and the montane regions of the Neotropics. Pennell (1934) made much the same sort of study of *Castilleja* in the western Nearctic, but with more limited material and lacking a full understanding of the Palearctic forms. While many unanswered questions remain in the analysis of *C. pallida* and its near relatives, some conclusions regarding the entire complex are now within reach, such as the synonymies presented in this and the three preceding new combinations. My research indicates that *C. pallida* is best viewed as a complex of largely parapatric varieties. While full explication of those occurring in the Palearctic awaits future field studies and examination of type specimens not easily accessible to me, I believe that *C. pallida* is represented in the Nearctic by three variable forms, the nominate variety, var. *caudata* (Pennell) B. Boivin, and var. *yukonis*. The latter two forms were treated as separate species by Pennell (1934). Neither of these is strongly divergent from the nominate variety, which appears to have a wide range across much of northern-central and northeastern Eurasia. Pennell believed that the nominate form also occurs in coastal northwestern Alaska. My examination of herbarium sheets supports this assertion, especially with collections from the general

vicinity of Nome, Alaska. However, further fieldwork in that region will be needed to clarify the status and distribution of var. *pallida* in North America.

Among the additional Nearctic taxa treated by Pennell (1934) and Hultén (1949) as part of the *Castilleja pallida* complex, I regard *C. annua* Pennell as synonymous with *C. pallida* var. *caudata*. The type collection of *C. annua* represents an inconstant morph with small flowers and branching stems found sporadically throughout much of the range of var. *caudata* and usually grading into the more typical morphs within populations and local areas.

The closely related taxa *Castilleja elegans* Malte and *C. raupii* Pennell are more strongly differentiated from *C. pallida* and deserve recognition as full species.

Castilleja pectinata M. Martens & Galeotti var. ***purpusii*** (Brandegee) J.M. Egger, **comb. et stat. nov.** BASIONYM: *Castilleja purpusii* Brandegee, Zoe 5: 181, 1904. TYPE: México. Est. de México: on rocky slopes above the timber line of [Volcán] Ixtaccihuatl [= Iztaccihuatl], Mar-Jul 1903, *Purpus 320* (holotype: UC!; isotypes: GH!, MO!, RSA-POM!, US!).

Comments: The taxon described as *Castilleja purpusii* is very similar to the earlier *C. pectinata*, but the vegetative characters originally used to distinguish the two are consistent in the plants found on the subalpine slopes of the Volcán Iztaccihuatl-Volcán Popocatépetl massif, to which var. *purpusii* appears to be endemic.

Castilleja rubicundula (Jeps.) T.I. Chuang & Heckard var. ***lithospermoides*** (Benth.) J.M. Egger, **comb. et stat. nov.** BASIONYM: *Orthocarpus lithospermoides* Benth., Scroph. Ind.: 13, 1835. *Castilleja rubicundula* subsp. *lithospermoides* (Benth.) T.I. Chuang & Heckard, Syst. Bot. 16: 658, 1991. TYPE: United States. (Nova) California: [probably in San Francisco Bay region or southwestern portion of Sacramento Valley], (1833) [1831,1832 or possibly Nov 1833], *Douglas s.n.* (holotype: K-BENTH!; isotypes: BM!, CGE!, GH!, PH!). [Note: the Kew Herbarium labels on some of the type sheets indicate the collection date as 1833, but this is probably incorrect, as Douglas was only in California from 4-28 November of that year, during which time it is very unlikely that this Spring annual would be in full bloom. It is

also possible that 1833 indicates when Bentham actually received the specimens at Kew. The isotype sheet at PH contains two different collections of this taxon, with the type collection represented only by a single stem mounted on the left side of the sheet, with a specimen from another collector on the right. The GH isotype sheet contains only a single stem of type material mounted on the far right portion of the sheet, along with stems from three other collections of the same species obtained by others mounted elsewhere.]

Castilleja subinclusa Greene var. ***jepsonii*** (Bacig. & Heckard) J.M. Egger, **comb. et stat. nov.** BASIONYM: *Castilleja jepsonii* Bacig. & Heckard, Leafl. W. Bot. 10: 282, 1966. TYPE: United States. California: San Benito Co., north-facing slope of side-cañon, among shrubs of *Haplopappus linearifolius*, just north of "Syncline Divide", in narrow valley of Vallecitos Creek, along road to New Idria, about 10.5 miles south of Panoche, elevation about 2000 feet, 14 May 1958, *Bacigalupi 6343, with Robbins & Hutchison* (holotype: JEPS!; isotypes: BM!, CAS!, F!, GH!, JEPS!, MICH!, NY!, OSC!, RSA!, US!, WTU!).

Castilleja exserta Eastw. ex C.F. Baker, W. Amer. Pl. 3: 4, 1904, nomen nudum. TYPE: United States. California: Tulare Co., S. Fork Kaweah River, Summer 1904, *Culbertson s.n.*, distributed as *Baker 4300* (holotype: CAS?; isotypes: K!, MO!, ND-G!). [Note: this name is spelled "*exerta*" by Eastwood on the isotype sheets.]

Comments: Although *Castilleja jepsonii* was subsumed within *C. subinclusa* by Chuang & Heckard (1993), the characters used by Bacigalupi & Heckard to first distinguish it from *C. subinclusa* at the species level appear to be consistent and sufficient to warrant recognition at the varietal level. In addition, var. *jepsonii* occurs in more open, brushy, and xeric habitats than does the uncommon nominate variety, which is limited to openings and borders in oak woodlands of the central-western foothills of the Sierra Nevada.

Castilleja tenuiflora Benth. var. ***tancitaroana*** (G.L. Nesom) J.M. Egger, **comb. et stat. nov.** BASIONYM: *Castilleja tancitaroana* G.L. Nesom, Phytologia 73: 401, 1992. TYPE: México. Michoacán: Mpio. Ziracuaretiro, 12 km NE of Uruapan, in San Andres Coru, pine-oak woods, "someros" soils in malpais, 1710 m,

24 May 1980, *Soto N. 2211* (holotype: TEX!; isotype: MEXU!).

Comments: This recently described taxon from central Mexico is distinguished by relatively minor but reasonably consistent features from typical *C. tenuiflora*. However, my field work across most parts of the described range indicates that the differences are not as extensive or consistent as portrayed in the protologue, and I believe that this form is better viewed as a morphological variant of *C. tenuiflora*. However, because it occurs in fairly uniform populations, with a definable center of distribution in Michoacán, I favor maintaining it at the varietal level. The two varieties are almost inseparable in the field, but the odd, mostly sunken glandularity of the foliage in var. *tancitaroana* is striking under magnification and unusual in the genus as a whole.

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OPTIMIZING PROCEDURES TO OBTAIN RELIABLE DNA FINGERPRINTING DATA FOR USE IN SYSTEMATIC, ECOLOGICAL AND EVOLUTIONARY STUDIES.

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ABSTRACT

The use of PCR based fingerprinting using short primers is very sensitive to variation in procedures. Strict laboratory procedures, exceeding general molecular biology standards, must be followed. Many laboratories have experienced difficulties in obtaining reproducible banding. Using a stock solution greatly minimizes pipetting small quantities, but the stability of a *Taq* stock is not well known. An investigation of the stability of a RAPDs stock (*Taq*, $MgCl_2$, 10X buffer, dNTPs) revealed the stock to be very stable (for 4 d, and for 60 d at 22°C). Interim storage of DNA at 4°C was found to be a significant source of variability in banding. Variability due to different amounts of polysaccharide inhibitors in the DNA is a significant source of variation in banding. Experiments show that diluting the DNA to about 0.25 ng/rxn (15 µl) dilutes the effects of inhibitors, yielding stable banding. Thermocycler temperature variation between runs can be a problem and methods for monitoring are presented. Other sources of variability are discussed and remedies suggested.

KEY WORDS: PCR, RAPDs, DNA fingerprinting, lab procedures

DNA fingerprinting methods (i.e., producing a bar-code of DNA bands) that utilize inverted repeats include RAPD (Random Amplified Polymorphic DNA), ISSR (Inter Simple Sequence Repeats) and SSR (Simple Sequence Repeats, when using a single primer). Most of these methods do not require sequence knowledge and are widely utilized in gene mapping, populational studies, infraspecific variation, cultivar identification, etc. Studies concerning higher levels of relationships (between genera, families, etc.) almost exclusively utilize DNA sequencing. A search of PubMed and SciFinder gave the following frequencies of citations : RFLP (PubMed 30,621; SciFinder 32,764), RAPD (4,343; 12,560), AFLP (1,597; 5,253), SSR (3,862; 12,659); ISSR (208, 807) and microsatellites (23,612; 7,961). Although RFLP has been the method of choice for gene mapping, other PCR based methods are finding considerable utilization.

RAPDs (Random Amplified Polymorphic DNAs) is a PCR technique that generates DNA fingerprints using a single oligonucleotide primer. The polymorphisms observed may result from point mutations, insertions, deletions and inversions (Williams, et al., 1990). RAPDs are usually dominant markers and are inherited in a simple Mendelian fashion. In comparison with RFLP, the procedure is less expensive, faster, requires a smaller amount of DNA (0.1-0.5 ng), does not involve the use of radioisotopes or fluorescent labels and requires less skill to operate. Because of these advantages RAPDs have proven useful in genotype identification and gene mapping as well as evolutionary studies (Demeke and Adams, 1994).

However, all PCR DNA banding methods rely on clean DNA, reproducible thermocycling temperatures, cycle times and stable, active *Taq* polymerase (or other DNA polymerase), exact pipetting of homogenous solutions and exceptional laboratory methods. Although RAPDs will be the focus of much of this paper, the results apply to other PCR based, fingerprinting methods. Obtaining reproducible RAPD patterns can present a problem in many labs. In fact, Penner et al. (1993) have investigated reproducibility in RAPDs using the same target DNA and primers in different laboratories. They found considerable differences between labs. They concluded that "if the overall temperature profiles (especially the annealing temperature)

inside the tubes are identical among the laboratories, then RAPD fragments are likely to be reproducible."

Benter et al. (1995) concluded that "a slow heating/ ramping from the annealing to the extension temperature increased the number of amplified bands and enhanced reproducibility". Yu and Pauls (1992) explored various PCR programming details to optimize the reactions for RAPDs production. Levi, Rowland and Hartung (1993) evaluated a range of concentrations of Triton X-100, gelatin, dNTPs, primer, template DNA, *Taq*, $MgCl_2$, as well as various times for annealing, elongation and denaturation. Bielawski, Noack and Pumo (1995) examined changes in protocols to obtain reproducible RAPD markers in vertebrate DNA (striped bass, plus a few other vertebrate species). In general, they found that using 30 sec denaturing and 30 sec. annealing times, coupled with the addition of a single-strand binding protein, Gp32, to the reaction mixture prevented nonspecific primer annealing during preparation of the reaction.

Han et al. (2003) examined the stability of RAPDs for genotyping *Helicobacter pylori* and reported that the method was very useful, however, "it seems unstable in amplification of low yield fragments, especially those that do not appear as visible bands on the agarose gel stained with EB, since the primer is partially matched to the template." Of course, these very low intensity bands would never be scored in systematic or evolutionary studies.

A recent examination (Adams, unpublished) of *Styrax texanas* revealed that there is essentially no variation among 30 individuals from three populations (Figure 1). Lanes 1-10, 11-20 and 21-30 are individuals from 3 geographically distinct populations. There is uniformity in the number and intensity of bands. Unless one has extremely good laboratory procedures, it is very difficult to obtain these kinds of results.

Adams, Flournoy and Pandey (1998) examined several sources of errors that cause difficulty in obtaining reproducible PCR amplification, even when using a single PCR machine. Non-uniform mixing of *Taq* was found to lead to considerable variation between duplicate runs. Because glycerol is included in the *Taq*, the material is

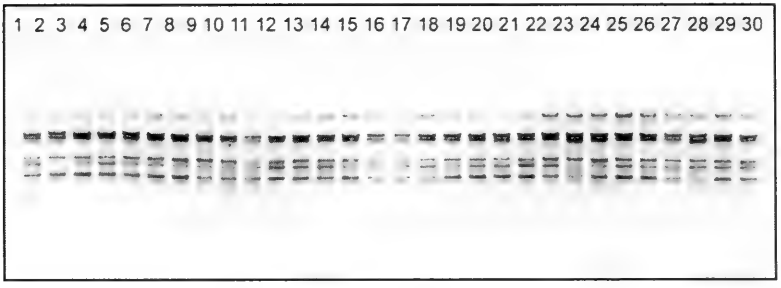

Figure 1. RAPD profiles for 30 individuals of *Styrax texanus* from 3 different populations (1-10, 11-20, 21-30) using UBC primer 431. Note the uniformity of the banding patterns.

difficult to mix and tends to settle to the bottom of the tube. Pipetting of small amounts is a significant source of errors. Therefore, stock solutions that include *Taq* should be prepared and aliquoted. In this paper, we expand on factors that cause difficulty and present some solutions to these problems involved in PCR for RAPDs.

MATERIALS AND METHODS

Plant material

Leaves were obtained from native plants (species, collection number): *Juniperus ashei* Buch. (Adams 7433, 7473), *J. flaccida* Schlecht. var. *flaccida* (Adams 6892), and *Prosopis glandulosa* Torr. (Adams 7375, 7401). Vouchers are on deposit at the Baylor University Herbarium (BAYLU). DNA was extracted using the hot CTAB protocol (Doyle and Doyle, 1987) (note: we recently began using the Qiagen DNeasy plant mini kit and have found it to be superior to the classical CTAB extraction for most species).

RAPD analysis

PCR was performed in a volume of 15 µl containing 50 mM KCl, 10 mM Tris-HCl (pH 9), 2 mM $MgCl_2$, 0.01% gelatin and 0.1% Triton x-100, 0.2 mM of each dNTPs, 0.36 µM primer, 0.3 ng of DNA (except as noted below), and 0.6 unit of Promega *Taq* DNA polymerase. The primers used in this study were (5'-3'): IBC 237: CGA CCA GAG C; #250 CGA CAG TCC C; #327 ATA CGG CGT C; #431 CTG CGG GTC A from the University of British Columbia.

Amplification was performed in a MJ Research Programmable Thermal Cycler. The thermal cycling was: 94°C (1.5 min) for initial strand separation, then 40 cycles at 38°C (2 min) for annealing, 72°C (2 min) for extension, 91°C (1 min). Two additional steps were used: 38°C (2 min) and 72°C(5 min) for final extension. Amplification products were analyzes by electrophoresis in 1.5% agarose (Sigma) gels and detected by staining with ethidium bromide.

Stability of RAPD stock

Because it is desirable to prepare a large volume of the RAPD stock, it is important to understand the stability of *Taq* and how long the stock can be stored. Preliminary tests, storing the *Taq* at 4°C, did not find any changes after 2 months. To hasten the changes, a new RAPD stock (ddwater, *Taq*, $MgCl_2$, 10X buffer, dNTPs and *Taq*) was made and stored at 22°C, then utilized after 4 days, 2 weeks, and after 60 days. In addition, a complete PCR RAPD mixture (stock + DNA + primer) was made and stored at 22°C for 4 days, 2 weeks, and 2 months before running PCRs.

Stability of DNA stored at 4°C

Often DNA is diluted to a uniform concentration (0.05 ng/µl). During the course of running many PCR reactions, DNA stocks are often stored at 4°C to save time in thawing them and avoid possible freeze-thaw problems. After a few months of DNA storage at 4°C, the loss of many bands was observed. When a new dilute DNA stock was made from DNA stored at -20°C, the original banding pattern was restored. To investigate the stability of diluted DNA, samples were stored at 4°C and -20°C, and the DNA analyzed after 1, 3, 5 and 8 months of storage. To investigate the effects of freeze/thaw cycles on DNA for PCR, frozen DNA (-20°C) was thawed every day, an aliquot of DNA taken, and a PCR run, then the DNA sample was re-frozen each day for 60 days.

Dilution of inhibitors by diluting genomic DNA

Many plant species produce considerable amounts of polysaccharides that are inhibitory to RAPDs PCR (Pandey, et al. 1996). *Juniperus flaccida* is such a species. PCR was performed using primer UBC 237 with various amounts of *J. flaccida* DNA: 1 ng/rnx., 0.5 ng/ rxn., 0.25 ng/rxn. and 0.125 ng/rxn. to examine these effects.

RESULTS AND DISCUSSION

Because errors in pipetting very small quantities are frequent, these errors could be minimized by preparing a large quantity of RAPD stock (ddwater, $MgCl_2$, 10x buffer, dNTPs and *Taq*). However, *Taq*, being an enzyme, might lose some activity when stored in solution. Preliminary storage of RAPD stock at 4°C did not reveal any differences after 2 months. In order to increase the rate of chemical reactions (degradations), the stock was stored at 22°C for various periods. Figure 2 shows that the RAPD stock is unaffected after 4 days at 22°C. It was also unaffected after 2 weeks (data not shown). However, after 60 days at 22°C, there is a noticeable decrease in band brightness, but the pattern and relative intensity of the bands is very similar to the control. Apparently the RAPD stock is very stable at 22°C and, also extremely stable when stored at 4°C (data not shown). However, when the primer and target DNA were included in the

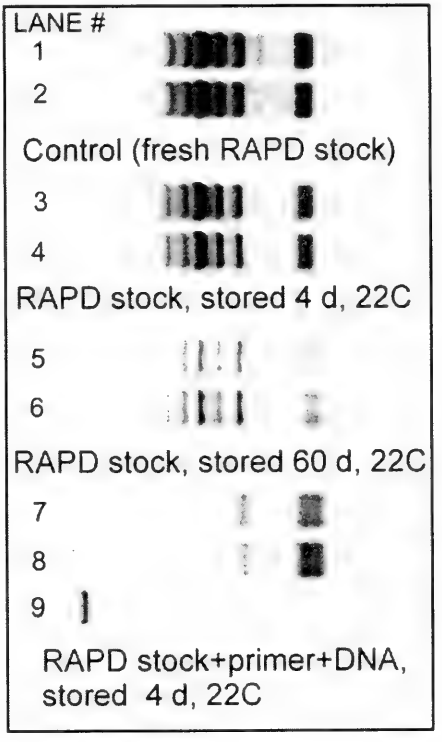

Figure2. PCR using primer 250 and *P. glandulosa* DNA (7401): Lanes 1,2 - fresh RAPD stock; Lanes 3,4 - RAPD stock stored 4 days at 22°C; Lanes 5,6 - RAPD stock stored 60 days at 22°C; Lanes 7,8 - PCR using RAPD stock plus primer 250, plus *P. glandulosa* DNA(7401) stored for 4 days at 22°C; Lane 9 - pGEM markers.

mixture, it was not stable after 4 days at 22°C (Figure 2). To reduce variation among analyses, it is recommended that an entire tube of *Taq* be used to make RAPD stock (ddwater, $MgCl_2$, 10x buffer, dNTPs and *Taq*) and the stock be stored at 4°C. Note that the loss of high molecular weight bands is an indication that the stock is degraded.

Due to the reproducibility problems encountered when storing DNA at 4°C (for ease of utilization), a systematic investigation of DNA storage temperature vs. PCR-RAPDs was performed. Figure 3 shows the triplicate analyses of DNAs from *Prosopis glandulosa* and *Juniperus ashei*, stored frozen (-20°C) vs. refrigerated (4°C). Notice that in every case, the frozen DNA yielded reproducible bands, whereas the refrigerated DNAs (stored for 8 mos.) showed considerable

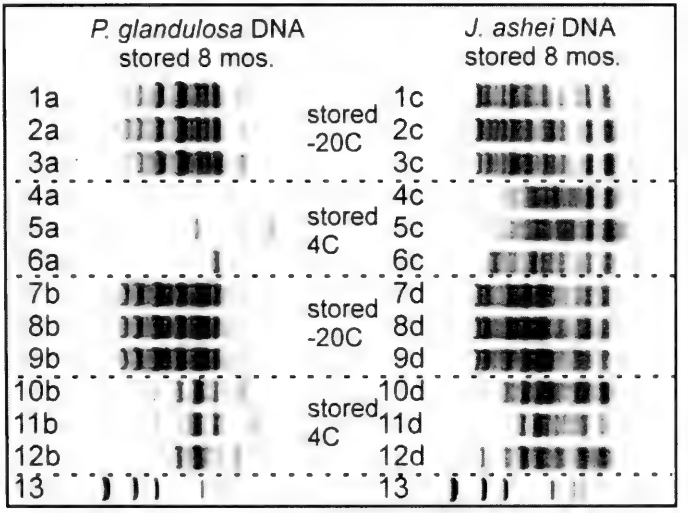

Figure 3. Comparisons of RAPDs (primer 327) obtained from frozen DNA vs. refrigerated DNA (4°C). Panel A: Lanes 1-6, *P. glandulosa* DNA (7401), lanes 1-3 - frozen DNA, lanes 4-6 - DNA stored for 8 mos. at 4°C; Lanes 7-12, *P. glandulosa* DNA (7375), lanes 7-9 - frozen DNA, lanes 10-12 - DNA stored for 8 mos. at 4°C. Panel B: Lanes 1-6, *J. ashei* DNA (7473), lanes 1-3 - frozen DNA, lanes 4-6 - DNA stored at 4°C for 5 mos.; lanes 7-12, *J. ashei* DNA (7433), lanes 7-9 - frozen DNA, lanes 10-12 - DNA stored at 4°C for 8 mos.

variability among the bands and a general loss of the higher molecular weight bands. Storage of DNA at 4°C for 2 weeks and one month did not reveal problems. Clearly, aliquots of genomic DNA can be stored at 4°C for several weeks. However, do several freeze-thaw cycles also affect the PCR-RAPDs? A sample of DNA was subject to 60 cycles (days) of freeze-thawing. Analysis revealed there were no effects on the RAPD pattern (data not shown). It seems likely that storing diluted for a few days at 4°C will not affect PCR. However, the loss of higher molecular weight bands is a clear indicator that the DNA is degraded and should be replenished with new DNA.

Polysaccharides have been shown to inhibit PCR and RAPD banding (Pandey, et al. 1995). It is likely that other inhibitors such as proteins and pigments may be in the DNA extract. Although several methods have been proposed to eliminate inhibitors, a general method is unlikely to be found that will work on all plants (or organisms). *Juniperus flaccida* is a species that produces PCR inhibitors. The potential of using dilution of the DNA to reduce the effects of inhibitors was examined. It is clear that 1 ng of DNA/rxn. is completely inhibitory for primer 237 (Figure 4). At 0.5 ng/rxn. the banding appears, but the highest molecular weight band is missing (the loss of high molecular weight bands is an excellent indicator of the presence of inhibitors). The banding is fully restored at 0.25 ng/rxn and 0.125 ng/rxn. (Figure 4). If high molecular weight bands are not obtained, one should dilute the DNA and re-run the PCR.

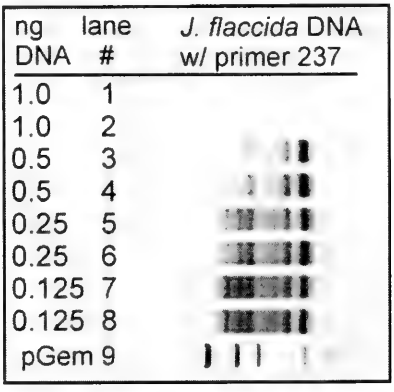

Figure 4. Effects of dilution of DNA from *Juniperus flaccida* (6892) on inhibition of PCR (primer 237). Lanes 1,2 - 1 ng/rxn., lanes 3,4 - 0.5 ng/rxn., lanes 5,6 - 0.25 ng/rxn., lanes 7,8 - 0.125 ng/rxn., lane 9 - pGEM markers.

Optimizing procedures to obtain reliable DNA fingerprinting data in systematic and evolutionary studies

There are several procedures that seem very important in obtaining reproducible RAPD banding and other PCR based fingerprinting. It is very important to vortex all reagents and DNA that have been frozen. This is critical for the Mg stock, as it tends to precipitate upon freezing/thawing, but this seems to be a potential problem with all components (e.g., primers, DNA, dNTPs, *Taq*, Mg, 10X buffer, etc.).

It is best to prepare an entire tube of *Taq* for the RAPD stock (but don't add a primer or DNA). The RAPD stock is stable at 4°C for several weeks. Freeze the RAPD stock if it will not be used for more than one month. Centrifuge the *Taq* tube before adding any components to get the *Taq* near the bottom of the tube. Centrifuge the tube again after all components (ddwater, $MgCl_2$, 10X buffer, dNTPs and *Taq*) have been added. Vortex to get the components well mixed, then centrifuge (but only for a pulse). Then repeat the vortex and centrifuge step. Mixing is very critical and this can be a major problem in training new students. Using a large RAPD stock solution will reduce errors and decrease variability in RAPD analyses

It is important to make up working stocks of DNAs (ex. 0.1 ng/µl), but these stocks should be stored at least at -20°C. It is recommended to return the working DNA stocks back to the freezer after thawing the working DNA stocks prior to making up the PCR reactions. Diluting the DNA in 1mM Tris (pH 8.5) is an effective way to prevent DNA degradation (data not shown).

Perform a concentration test on your DNAs with a reliable, proven primer. Run your DNA at the lowest concentration possible where you still get good, bright bands. For *Juniperus*, 0.3 ng of DNA / 15 µl PCR reaction has effectively eliminated problems with indigenous inhibitors. However, it was necessary to use 0.15 ng of DNA / 15 µl PCR reaction for *Prosopis* analyses.

Proteinase is not often included in most plant DNA extraction protocols, but we have found it to be essential for the extraction of alcohol-preserved specimens (Flournoy, et al. 1996). If extracts of alcohol-preserved specimens are not incubated in proteinase, the histones that have been precipitated onto the DNA will result in the loss of the DNA during extraction. Although Proteinase K is listed for

many protocols, one can substitute Pronase E and it costs only about 5% the cost of Proteinase K.

Try different methods for grinding materials. For most plants, grinding fresh leaves in hot CTAB (60°C) or extraction buffer, resulted in higher yields and higher molecular weight DNA than grinding the leaves in liquid nitrogen and then placing the ground material in hot CTAB (or extraction buffer). However, Adams, Zhong and Fei (1999) reported that for *Vetiveria*, a tropical grass (and all other grasses examined), the DNA yield was very small and it was almost completely degraded when either fresh or dried leaves were ground in hot CTAB (or an extraction buffer). However, grinding the leaves in ethanol resulted in good yields and high quality DNA. We now grind all plant samples in as small amount of ethanol as possible, then dilute with extraction buffer before treating with proteinase (if too much ethanol is present, proteinase will be inhibited). The Qiagen mini-plant extraction kit is also excellent for most applications, but it is very difficult to use on ethanol preserved materials.

The maximum temperature (ex. 94°C) and minimum temperature (ex. 40°C) for each well of the Thermocycler should be checked. You may find some cool spots around the margin of the heating block. If so, do not use these areas of the plate.

The temperature pattern for each PCR run should be monitored with a linear strip chart recorder (e.g. Cole Parmer 201 chart recorder coupled to a Omega Engineering, CJ cold joint temperature compensator). This will generate an exact record of each temperature cycle for every run (Figure 5). If there is a deviation in temperature (maximum, minimum or cycle width), it will be very evident. If

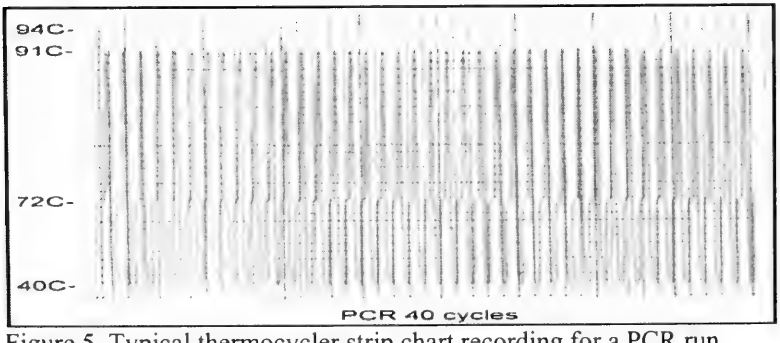

Figure 5. Typical thermocycler strip chart recording for a PCR run.

deviations occur, the PCR thermocycler must be repaired and re-calibrated. A copy of the temperature profile can be placed in the lab book with each PCR analysis.

One of the best safeguards is to run two very closely (or identical) individuals for each taxon or population. Figure 6 shows 5 taxa of *Juniperus* with 2 individuals run from each taxon. Two similar individuals that were growing near each other were intentionally sampled and used to represent the taxon. Notice that each of the pairs is very similar in their banding pattern (Figure 6). It is often the case that one of these will not have the larger band or the larger bands will be very faint. One should re-run DNA in triplicate from the poor performing individual. If it still fails to be similar to the other individual, after diluting the DNA, then new DNA needs to be extracted. Variation in banding between near-identical individuals (or sibs) is an excellent method by which to obtain constant feedback in every analysis.

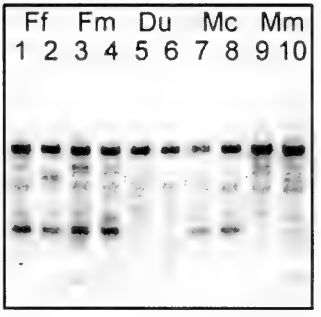

Figure 6. DNA banding for pairs of individuals from five taxa of *Juniperus*. Note the similarity between individuals.

After loading all components into a PCR tube for a RAPD run, it is important to make sure all the mixture is in the bottom of the tube (pulse centrifuge in a mini-centrifuge), then vortex 10 sec to make a uniform mixture. The *Taq* tends to settle on the bottom and it is critical that the *Taq* be well dispersed in the solution. A quick pulse centrifuge of the mixture is required to get all the solution to the bottom of the tube. Then check to see if any air bubbles are in the tube. If there are bubbles in the tube, tap it on a table until they are removed, then centrifuge with a quick pulse. This is such a critical procedure that we do this twice to make very sure that components are mixed.

If you are using a PCR tube format, add a drop of oil to the holes in the PCR machine. When you place the PCR tube in a hole, a little oil should come out around the tube. Be sure tubes fit down securely in the holes in the PCR machine and that the PCR tube lids are completely closed.

Evaluate PCR tubes from several suppliers, using the same DNA, primer(s), *Taq*, etc. Use the PCR tubes that work best for your PCR machine and lab conditions. There are considerable differences among suppliers.

Screen lots of primers. Screening of 600 primers from the University of British Columbia has revealed (Adams, et al. 1998) that about 25% of the primers give no bands, about 50% give a few bands and about 25% produce 5 or more bands. Of the best 25%, about 1/4 will be really excellent. Out of 100 primers, one should expect to find 5 to 10 really good primers. Generally, these primers are useful for all species we have studied (ranging from gymnosperms to monocots and dicots, as well as fish). It is not time-efficient to attempt to optimize the PCR (Mg concentration is particularly sensitive) for every possible primer.

Some primers are more useful at the specific level, whereas other primers are more useful to characterize differences between individuals. Screen until primers are found that will give the necessary resolution.

In addition, it should be emphasized that multivariate statistical methods have the capability of accounting for error variance and are highly desirable for analysis. The use of parsimony tree building methods is not appropriate for PCR based methods because there is no provision to allow for error variance. However, chemosystematists, who have worked many years with secondary compound data, are well aware of error variance and the need to factor data to remove (and account for) error variance. Multivariate methods that are compatible with PCR banding data include PCO (Principal Coordinate Ordination), PCA (Principal Components Analysis), and CVA (Canonical Variate Analysis). Minimum spanning networks and neighbor joining methods can be used with some cautions.

In conclusion, obtaining reproducible PCR banding can be difficult. It demands very strict lab conditions and attention to detail. However, PCR banding can reproducible if exacting laboratory procedures are followed and appropriate analyses methods are utilized.

ACKNOWLEDGEMENTS

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DISTRIBUTION OF *JUNIPERUS ASHEI* VAR. *ASHEI* AND VAR. *OVATA* AROUND NEW BRAUNFELS, TEXAS

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ABSTRACT

The distribution of *J. ashei* var. *ashei* and *J. a.* var. *ovata* was examined by leaf essential oils. The populational boundries between these varieties seems to be very distinct. However, there appears to be some intergradation between the two taxa near New Braunfels, Texas.

KEY WORDS: *Juniperus, J. ashei* var. *ovata,* , essential oils, distribution, Cupressaceae

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Juniperus ashei is a small tree that grows abundantly on limestone on the Edwards plateau in central Texas with disjunct populations on limestone in Arkansas, Missouri, and Oklahoma as well in Coahuila, Mexico (Fig. 1). The Edwards Plateau (limestone) region of central Texas supports dense populations covering millions of acres, whereas the disjunct populations (Fig. 1) often have almost pure stands of *J. ashei*, that may cover only a few acres.

Studies of geographic variation in *Juniperus ashei* have shown that the species has divergent populations in the semi-arid margins of its range (Adams, 1977, 2004). The divergent populations were recently recognized as a new variety (*J. a.* var. *ovata*, Adams and Baker, 2007). The leaf oils of *J. a.* var. *ashei* and *J. a.* var. *ovata* differ mostly quantitatively (Adams and Baker, 2007). Camphor is considerably larger in var. *ashei* (69.1%) than in var. *ovata* (53.5%). In contrast, bornyl acetate is must larger in var. *ovata* (15.6%) than in var. *ashei* (6.3%). Four (non-trace) compounds differ qualitatively(Adams and Baker, 2007): trans-sabinene hydrate, trans-p-menth-2-en-1-ol, verbenone, and sandaracopimara-8(14),15-diene. Several other compounds differ quantitatively: α-pinene, myrcene, p-cymene, limonene, γ-terpinene, linalool, trans carveol, carvone and elemol (Adams and Baker, 2007).

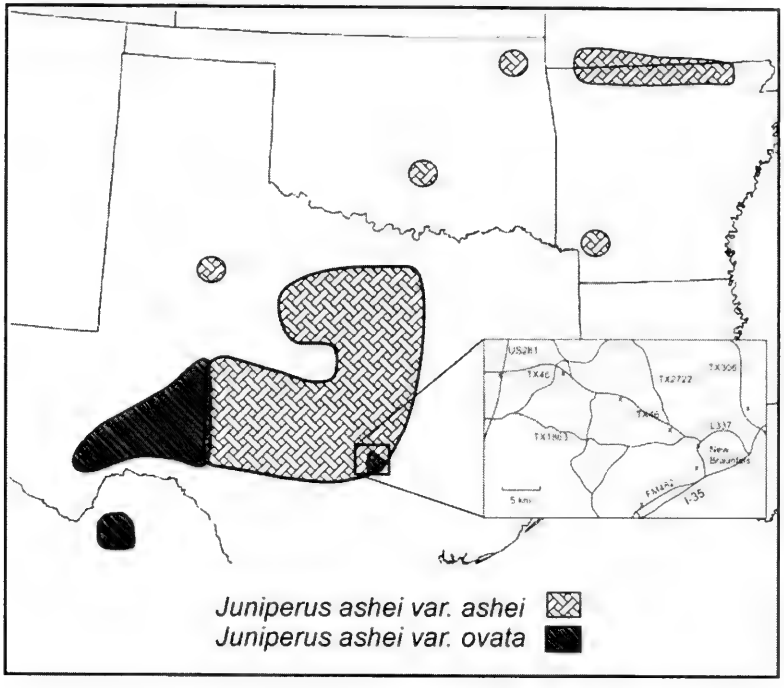

Figure 1. Distribution of *J. ashei*, adapted from Adams and Baker (2007). The study area is indicated by the fly-out box.

In the original study (Adams, 1977), the New Braunfels population of *Juniperus ashei* var. *ovata* was represented by samples from 15 individuals from a single population 8 km west of New Braunfels. The nearest populations sampled (Adams, 1977) were at Bandera and Hyde (80 - 100 km w and nw of New Braunfels) and these were typical *J. a.* var. *ashei*. Thus, it is not clear if var. *ovata* might extend further west.

The purpose of this study was to collect samples near New Braunfels, Texas to determine more precisely the range of vars. *ashei* and *ovata* in this region of disjunct populations.

MATERIALS AND METHODS

Specimens used in this study: *Juniperus ashei var. ashei*: Comal Co., TX: jct TX46 & US 281, *Adams 11295, 11296, 11297*; on TX 46, 8 km e of jct TX 46 and US 281, *Adams 11298, 11299, 11300*; on TX 46, 16 km e of jct TX 46 and US 281, *Adams 11301, 11302, 11303,* on TX 46, 24 km e of jct TX 46 and US 281, *Adams 11304, 11305, 11306, 11307, 11308,* on TX 306, 1 km nw of Hunter Rd, *Adams 11322, 11323, 11324. J. a.* var. *ovata*: Comal Co., TX, Loop 337, 1 km s of jct TX 46 and Loop 337, *Adams 11314, 11315, 11316,* 40 m w of jct Cedar Elm St. and Madeline St. on Madeline St. (site of the National Big Tree for *J. ashei*), New Braunfels, *Adams 11309, 11317, 11318*, 100 m n of jct Hubertus Rd. and FM 482 on FM 482, *Adams 11319, 11320, 11321.* Voucher specimens are deposited at Baylor University (BAYLU).

Fresh leaves (200 g. fresh wt.) were steam distilled for 2 h using a circulatory Clevenger apparatus (Adams, 1991). The oil samples were concentrated (ether trap removed) with nitrogen and the samples stored at -20°C until analyzed. The extracted leaves were oven dried (48h, 100°C) for determination of their oil yields.

The essential oils were analyzed on a HP5971 MSD mass spectrometer, directly coupled to a HP 5890 gas chromatograph, using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column (see Adams, 2006 for operating details). Identifications were made by library searches of our volatile oil library (Adams, 2006), using the HP Chemstation library search routines, coupled with retention time data of authentic reference compounds. Quantitation was by TIC.

RESULTS AND DISCUSSION

Because tricyclene is fairly constant in *J. ashei*, by merely examining if the height of the α-pinene peak (that runs just after tricyclene on DB-5) is greater than tricyclene, one can determine that the oil is from var. *ovata*, whereas if α-pinene is less than tricyclene, the oil is from var. *ashei*.

Figure 2 shows that the samples taken along TX 46 from US 281 to near loop 337 are all the low in α-pinene. This is typical for var. *ashei*. The samples from loop 337 (L) are high in α-pinene that is typical of var. *ovata*. The samples of var. *ovata* from the National Big Tree site (N) are uniformly high in α-pinene. Two of the samples on FM 482 are typical var. *ovata*, but the third sample is more like var. *ashei*.

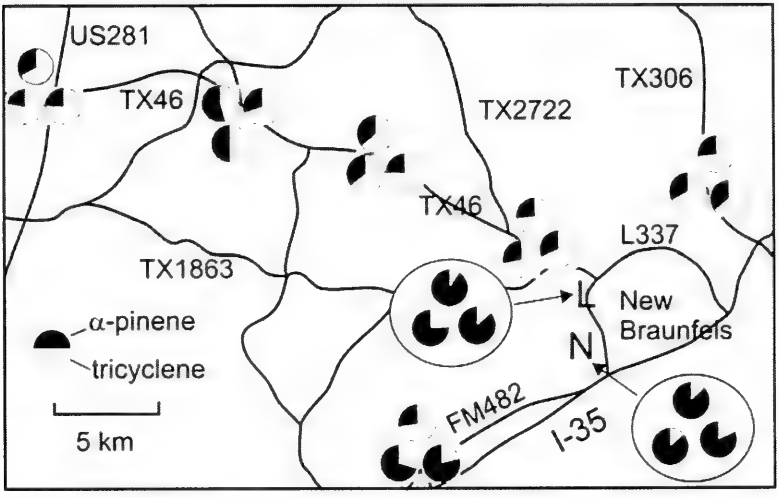

Figure 2. Distribution of *J. ashei* var. *ashei* and var. *ovata* based on the concentration of tricyclene and α-pinene.

Geographic variation in camphor and bornyl acetate show the same pattern (Fig. 3). However, at least one individual in both the FM 482 and the TX 306 populations appear to be intermediate between var. *ashei* and var. *ovata*.

This study shows that var. *ashei* and var. *ovata* co-occur at the lower elevation the Edwards plateau at New Braunfels. Additional sampling is needed to further define the distribution of var. *ovata* in this region.

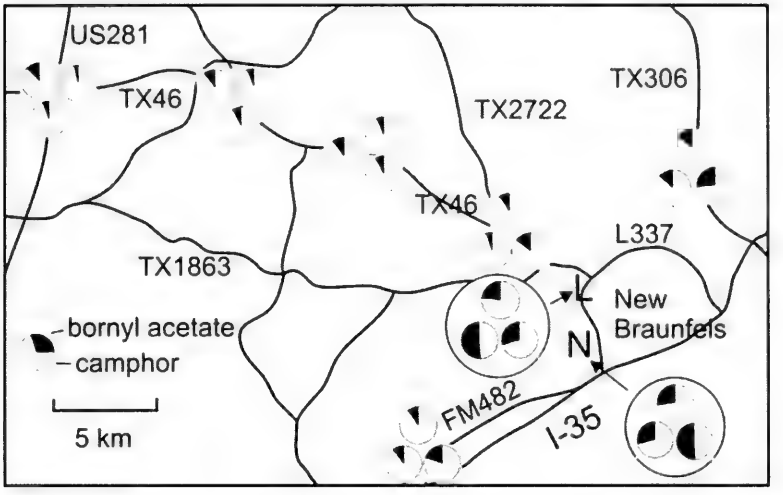

Figure 3. Distribution of *J. ashei* var. *ashei* and var. *ovata* based on the concentration of bornyl acetate and camphor.

ACKNOWLEDGEMENTS

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THE EVOLUTION OF CARIBBEAN *JUNIPERUS* (CUPRESSACEAE): TERPENOIDS, RAPDS AND DNA SNPs DATA

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ABSTRACT

SNPs from nrDNA and trnC-trnD cp DNA for the Caribbean junipers have provided additional insight into the evolution of this group of smooth leaf margined junipers. Comparing leaf terpenoids, RAPDS and SNPs from nrDNA and trnC-trnD cp DNA show that each data set reveals different facets of the relationships. In general, the data sets support four major groups: *Juniperus barbadensis - lucayana; J. bermudiana*; *J. gracilior - ekmanii - urbaniana*; *J. saxicola*; *J. virginiana - silicicola*. Based on the concordance of these data and morphology, the Caribbean junipers are treated as four species, three of these having varieties: *J. barbadensis, J. barbadensis* var. *lucayana, J. bermudiana, J. gracilior, J. gracilior* var. *ekmanii, J. gracilior* var. *urbaniana, J. saxicola, J. virginiana* and *J. virginiana* var. *silicicola*. The evolutionary colonization pathways of the Caribbean junipers are discussed.

KEY WORDS: *Juniperus,* Caribbean, evolution, systematics, terpenoids, RAPDs, nrDNA, trnC-trnD cp DNA, SNPs, Cupressaceae

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The Caribbean junipers have been the focus of our lab in several studies (Adams, 1983, 1986, 1989, 1995, 1997, 2000; Adams et al. 1987; Adams and Hogge, 1983). There are numerous older studies, beginning with Linnaeus (1753) who described only three junipers from the New World (*J. virginiana*, "Virginia and Carolina"; *J. barbadensis*,

"America"; and *J. bermudiana*, "America"). However, Hemsley (1883) equated *J. barbadensis* L. with *J. bermudiana* L., adopting *J. bermudiana* as the name for all of the Caribbean junipers. Sargent (1902) recognized *J. barbadensis* and said it occurred along the Atlantic coast of Georgia and Florida as well as "on the Bahamas, San Domingo (Dominican Republic), mountains of Jamaica and on Antigua." Britton (1908) recognized *J. lucayana* Britt. in the Bahamas and reserved *J. barbadensis* for the plants of southern Georgia, Florida, and the rest of the Caribbean. Pilger (1913) equated *J. bermudiana* and *J. barbadensis*, but used *J. barbadensis* for the name of the common juniper of the Caribbean on the grounds that it was listed first by Linnaeus (1753). Florin (1933) reviewed the junipers of the Caribbean and recognized 5 species: *J. saxicola* Britt. & P. Wilson from Cuba; *J. lucayana* from Cuba, Haiti, Jamaica and the Bahamas; *J. gracilior* Pilger from Haiti and Dominican Republic; *J. ekmanii* Florin from Haiti; and *J. urbaniana* Pilger & Ekman from Haiti. Carabia (1941) recognized *J. barbadensis* throughout the Caribbean, *J. bermudiana* on Bermuda, and *J. virginiana* in the United States. Gillis (1974) treated the Bahamian junipers as *J. bermudiana*. Correll and Correll (1982) recognized the juniper of the Bahamas as *J. barbadensis*.

Adams (2000, 2004) recognized: *J. bermudiana* (endemic to Bermuda); *J. barbadensis* (endemic to St. Lucia, extinct on Barbados); *J. lucayana* (Bahamas, Cuba, Jamaica, likely extinct in Haiti), *J. gracilior* (endemic to Hispaniola), *J. gracilior* var. *ekmanii* (Florin) R. P. Adams (endemic to Hispaniola), *J. gracilior* var. *urbaniana* Pilger & Ekman) R. P. Adams (endemic to Haiti), and *J. saxicola* (endemic to Cuba). The juniper of the southeastern United States that occurs on coastal foredunes was recognized as *J. virginiana* var. *silicicola* (Small) E. Murray (Adams, 2004). Farjon (2005) followed Adams (2004) treatment, except he recognized *J. lucayana* as *J. barbadensis* var. *lucayana* (Britt.) R. P. Adams.

Figure 1 shows the populations sampled in this and previous studies. Several taxa are very rare: *J. barbadensis* var. *barbadensis*, known only from Petit Piton, St. Lucia; *J. bermudiana*, endemic to Bermuda; *J. gracilior* var. *ekmanii*, Pic la Selle, Haiti and adjacent mountain in Dominican Republic. *J. gracilior* var. *urbaniana*, Pic le Selle, Haiti, *J. saxicola*, Pico Turquino, Cuba. *Juniperus barbadensis*

has been extinct on Barbados for over 280 years (Adams, 2004) and *J.* lucayana (*J. barbadensis* var. *lucayana*) is presumed extinct in Haiti (and Hispanola) (Adams, 2004).

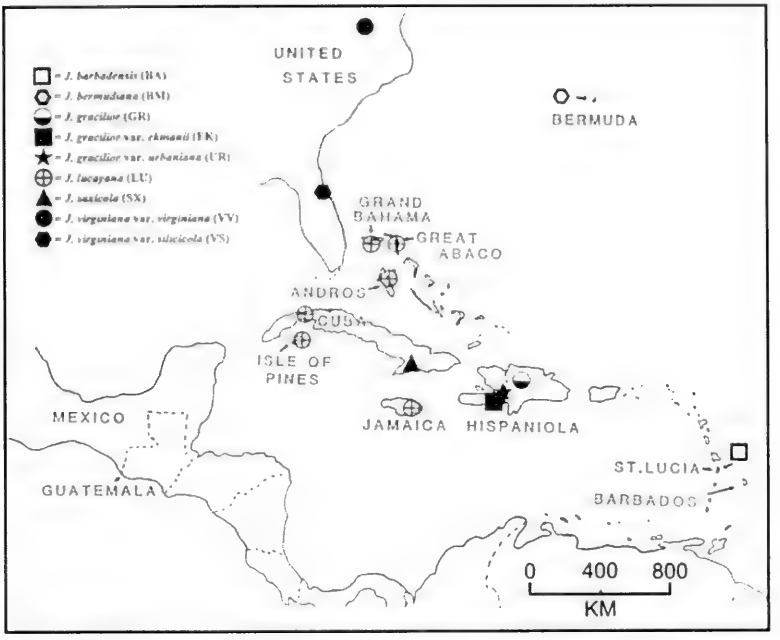

Figure 1. Populations of *Juniperus* sampled in this and previous studies.

Using terpenoid data (based on Adams, 1997), a PCO of the Caribbean junipers (Fig. 2) shows that all of the varieties of *J. gracilior* are very similar in their oils with similarities of 0.85 to 0.88 (eg. 85 - 88%). Their oils are dominated by bornyl acetate (35.7 - 43.9%, vs. less than 4.1% in all other taxa) and all have 0.5 - 0.9% cis-pinene hydrate (found only as a trace in *J. barbadensis*) and 0.8 - 1.2% α-terpineol (found only as a trace in all other taxa). It is interesting to note that the oil of *J. bermudiana* is quite distinct being only 0.77 similar to *J. lucayana* (Fig. 2) and much less similar to *J. virginiana* (0.63, not shown). *Juniperus barbadensis* (St. Lucia) is linked (0.82) to

J. lucayana (Cuba) and provides the linkage between *J. gracilior* (Hispanola) and *J. lucayana* (Cuba).

Juniperus virginiana var. *virginiana* and var. *silicicola* are very similar (0.83) and quite distinct, linking with *J. lucayana* (Bahamas) at 0.72. The *J. lucayana* samples are in two groups: the Bahamas and Cuba-Jamaica. This seems to represent a geographic split within their populations. *Juniperus saxicola* is loosely linked to *J. lucayana* (0.78, Fig. 2).

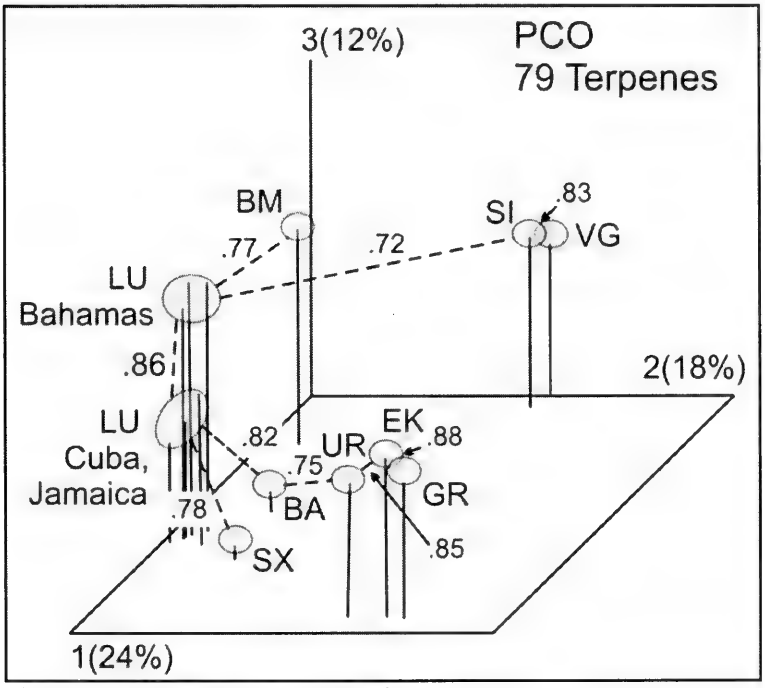

Figure 2. PCO based on 79 terpenoids.

Figure 3 shows the PCO based on 132 RAPDs (re-drawn from Adams, 2000). Again, one sees that all of the varieties of *J. gracilior* are very similar in their RAPDs with similarities of 0.83. *Juniperus bermudiana* is less distinct, and similar to *J. lucayana* (Fig. 3). Again, *J. bermudiana* is much more similar to *J. lucayana* (0.83) than to *J. virginiana* (0.70, not shown). This suggests that *J. bermudiana* did not arise from *J. virginiana* (directly) but from *J. lucayana* (or an ancestor).

With the RAPDs, *J. saxicola* links to *J. barbadensis*, but it has essentially the same linkage to *J. gracilior var. ekmanii* (not shown).

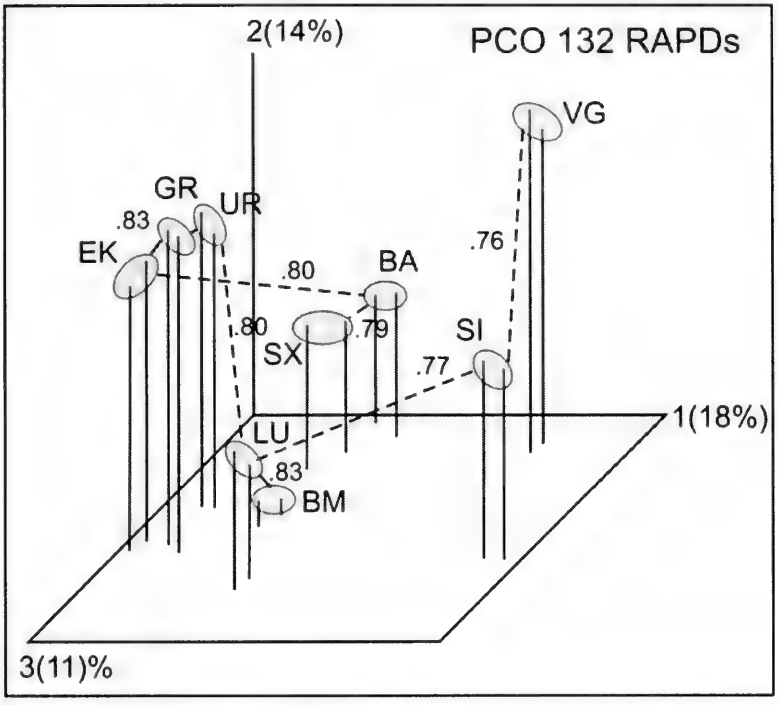

Figure 3. PCO based on 132 RAPDs.

Juniperus virginiana and *J. v.* var. *silicicola* are well separated (Fig. 3).

The purpose of this study was to utilize additional data sets (Single Nucleotide Polymorphisms, SNPs) of nr DNA and trnC-trnD, cp DNA to gain additional insight on the relationships among the *Juniperus* taxa in the Caribbean.

MATERIALS AND METHODS

Specimens collected: taxon, acronym, collector number, location: *J. barbadensis* (BA), *Adams 5367-5371;* Petit Piton, St.

Lucia, BWI; *J. bermudiana* (BM), *Adams 11080-11082*, Bermuda; *J. gracilior* var. *ekmanii* (EK), *Adams 7653-7654*, 3-4 km ne Mare Rouge, Pic la Selle, Haiti; *J. gracilior* var. *gracilior* (GR), *Adams 7664-7667,* w of Constanza, Dominican Republic; *J. gracilior* var. *urbaniana* (UR) *Adams 7656-7658,* 4-5 km ne Mare Rouge, Pic la Selle, Haiti; *J. lucayana: Adams 5259-5280*, Havana Botanical Garden (seed from Sierra de Nipe), Cuba; *Adams 5281-5282*, Havana Botanical Garden (seed from Isle de Pinos), Cuba; *J. saxicola* (SX) *Adams 5284-5285,* w slope of Pico Turquino, Prov. Granma/ Santiago de Cuba boundary, Cuba; *J. virginiana* var. *virginiana* (VG) *Adams 6753-6755*; on I35, Hewitt, TX; *J. virginiana* var. *silicicola* (SI) *Adams 9186-9188,* Ft. Desoto Park, Mullet Key, Florida. Herbarium vouchers for all of the aforementioned collections are deposited at BAYLU.

One gram (fresh weight) of the foliage was placed in 20 g of activated silica gel and transported to the lab, thence stored at -20° C until the DNA was extracted. DNA was extracted using the Qiagen DNeasy mini kit (Qiagen Inc., Valencia CA).

SNPs obtained from DNA sequencing

ITS (nrDNA) and trnC-trnD amplifications were performed in 50 µl reactions using 10 ng of genomic DNA, 3 units Qiagen Taq polymerase, 5 µl 10x buffer (final concentration: 50 mM KCl, 10 mM Tris-HCl (pH 9), 0.01% gelatin and 0.1% Triton X-100), 1.75 mM $MgCl_2$, 20 µl Q solution (2X final), 400 µM each dNTP, 1.8 µM each primer and 4%(by vol.) DMSO.

Primers (5'-3'):

ITS: ITSA = GGA AGG AGA AGT CGT AAC AAG G;
ITSB = CTT TTC CTC CGC TTA TTG ATA TG.

ITSA and ITSB primers from Blattner (1999).

trnC-trnD: CDFor: CCA GTT CAA ATC TGG GTG TC
CDRev: GGG ATT GTA GTT CAA TTG GT

CDFor, CDRev primers from Demesure et al. (1995).

CD10F: AAA GAG AGG GAT TCG TAT GGA
CD3R: AAC GAA GCG AAA ATC AAT CA

CD10F and CD3R primers from Andrea Schwarzbach (pers. comm.).

The following PCR conditions were used: MJ Research Programmable Thermal Cycler, 30 cycles, 94°C (1 min.), 50°C (2 min.), 72°C (2 min.), with a final step of 72°C (5 min.). The PCR reaction was subjected to purification by agarose gel electrophoresis (1.5% agarose, 70 v, 55 min.). The nrDNA primers (ITSA, ITSB) produced a band of approx. 1120 bp. The internal trnC-trnD primers, CD10F-CD3R produced a band of approx. 800 bp. In each case the band was excised and purified using a Qiagen QIAquick gel extraction kit.

The gel purified DNA band with the appropriate primer was sent to McLab Inc. for sequencing. Sequences for both strands were edited and a consensus sequence was produced using Chromas, version 2.31 (Technelysium Pty Ltd.). Alignments were made using Clustal W and then manually corrected. Indels were coded with a "-" for the first nucleotide and "I" for succeeding nucleotides such that an indel was treated as a single mutation event. Overall sequences have been deposited in GenBank (Schwarzbach et al., in prep.).

SNPs analyses

Aligned data sets (nrDNA and trnC-trnD) were analyzed by CLEANDNA (Fortran, R. P. Adams) to remove invariant data. Mutational differences were computed by comparing all SNPs, divided by the number of comparisons over all taxa (= Gower metric, Gower, 1971; Adams, 1975). Principal coordinate analysis was performed by factoring the associational matrix using the formulation of Gower (1966) and Veldman (1967). A minimum spanning network was constructed by selecting the nearest neighbor for each taxon from the pair-wise similarity matrix, then connecting those nearest neighbors as nodes in the network (Adams, 2004).

RESULTS AND DISCUSSION

Analyses of 1119 bp of nrDNA (ITS) sequences revealed 27 SNPs among the taxa including a 3 bp deletion in both samples of *J. g.* var. *ekmanii* and a 1 bp insertion in all six samples of *J. v.* var. *virginiana* and *J. v.* var. *silicicola*. PCO of the SNPs resulted in 5 eigenroots that were larger than the average diagonal value. These 5 eigenroots accounted for 42.49, 20.48, 12.98, 8.54 and 5.80% of the variation among the OTUs or a total of 90.21%. From this factor

analysis there appear to be 4 major and 2 minor groups. Ordination (Fig. 4) shows four major groups: (SI, VG, *J. virginiana*) , (BA, LU, *J. barbadensis - lucayana*), (BM, *J. bermudiana*), and (SX, UR, GR, EK,

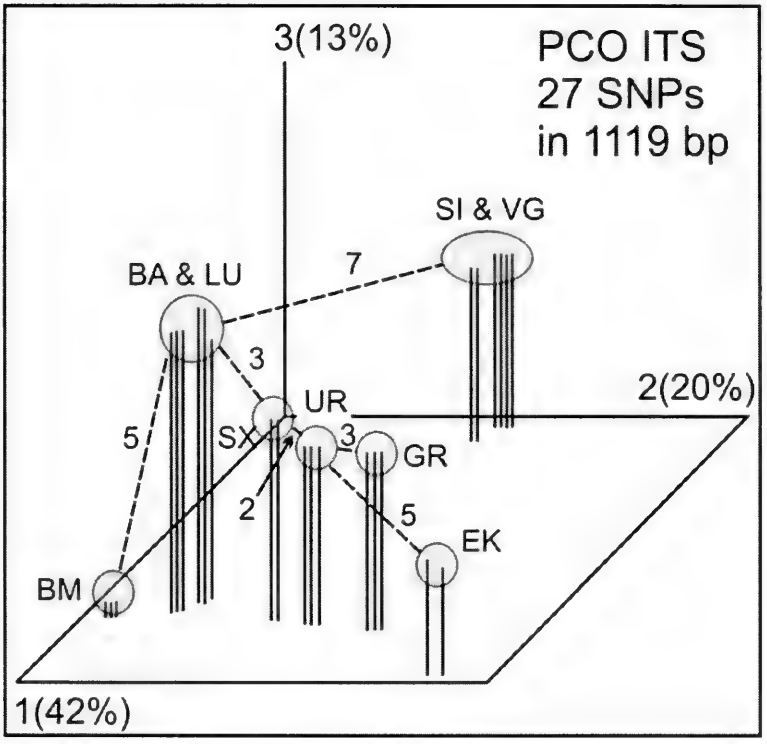

Figure 4. PCO based on 27 SNPs of nr DNA.

J. saxicola - J. gracilior). The most surprising facet is the 5 SNPs differentiating EK (*J. gracilior* var. *ekmanii*), from *J. gracilior* var. *urbaniana* (UR). Another interesting point is the very close linkage of *J. saxicola* with *J. gracilior* var. *urbaniana* and *J. barbadensis*. Because *J. saxicola* is frozen in neoteny and thus, has only juvenile leaves, it appears morphologically very distinct from all other Caribbean junipers. However, this may be misleading, as the SNPs indicate. *Juniperus bermudiana* is quite distinct.

Another aspect shown is that *J. barbadensis* (St. Lucia) and *J. lucayana* (Cuba) differed by only a single SNP. All samples of *J. barbadensis* were identical. The lack of variation is indicated in figure 4 in which identical sequences are denoted by vertical bars that are closely spaced. In fact, the only other polymorphisms were found in *J. g.* var. *ekmanii* (1 bp difference), and *J. v.* var. *silicicola* (2 bp). One *J. v.* var. *silicicola* was identical to *J. v.* var. *virginiana* (Fig. 4).

Sequencing of the trnC-trnD region of cp DNA with the CD10F - CD3R primers resulted in a partial sequence of 798 bp in *J. v.* var. *virginiana.* However, all the other taxa in this study had a 245 bp deletion resulting in 553 bp. Analyses of these sequences revealed 6 SNPs plus the deletion (coded as a single event or SNP). PCO of these data resulted in two eigenroots accounting for 100% of the variance among taxa with roots of 83 and 17%. Two eigenroots imply n+1 groups, or 3 groups of taxa. This is shown in figure 5. There was no variation found within groups (VG), (BA, BM, GR, LU, SI) and (EK, SX, UR). *Juniperus virginiana* (VG) from the mainland (and thought to be an ancient species, Adams, 2004), yielded 798 bp with this set of trnC-trnD primers and this size is consistent with the other smooth leaf margined junipers of the western hemisphere (Schwarzbach, et al., in prep.). However, *J. v.* var. *silicicola* shares the 245 bp deletion with all the junipers of the Caribbean. This seems to provide evidence that the Caribbean (plus Bermudian) junipers were derived from *J. v.* var. *silicicola* or a common ancestor or perhaps that *J. v.* var. *silicicola* was of hybrid origin between *J. lucayana* and *J. v.* var. *virginiana.*

Juniperus barbadensis (BA), *J. bermudiana* (BM), *J. gracilior* (GR), *J. lucayana* (LU), and *J. virginiana* var. *silicicola* (SI) share no SNPs (Fig. 5). It is notable that *J. saxicola* (SX) is grouped with *J. gracilior* var. *ekmanii* (EK) and *J. gracilior* var. *urbaniana* (UR) (Fig. 5). All members of the (EK, SX, UR) group share the single SNP that separates it from the (BA, BM, GR, LU, SI) group. The grouping of *J. saxicola* with *J. gracilior* var. *urbaniana* was also supported by the ITS SNPs (Fig. 4).

There is no doubt that the Caribbean junipers are extremely similar and very difficult to separate into species in the herbarium.

Figure 6 shows a diagram based on the gross morphology. *Juniperus bermudiana* and *J. saxicola* are the only taxa that are easy to key out.

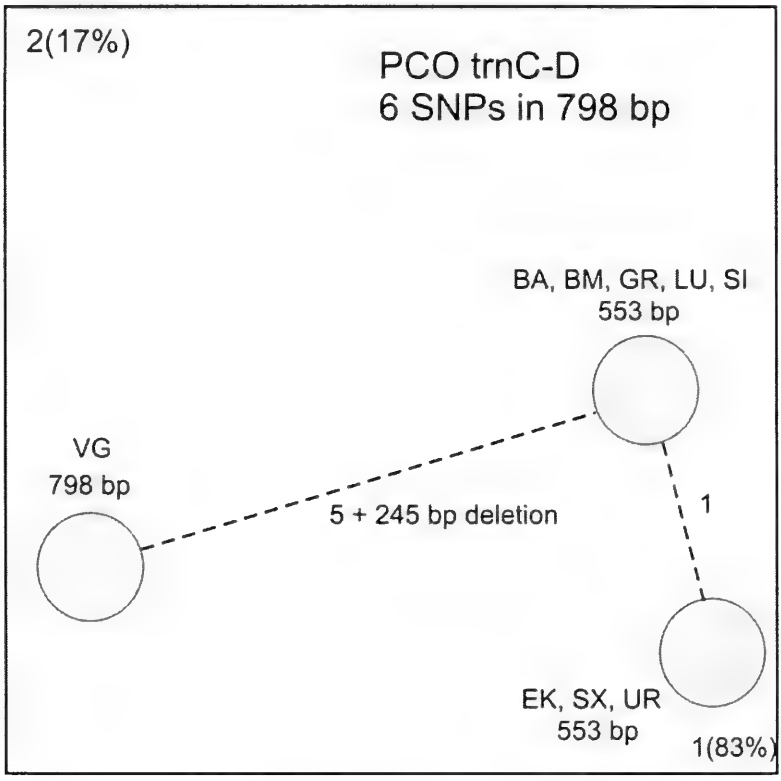

Figure 5. PCO of trnC-trnD SNPs based on 789 bp of sequence data. Note that there was no variation found in groups (VG), (BA, BM, GR, LU, SI) and (EK, SX, UR).

Juniperus bermudiana has opposite leaves that result in square leafy branchlets and *J. saxicola* has only awl shaped leaves due to the taxon being fixed in neoteny. However, examination of individuals at the summit of Petit Piton, St. Lucia revealed a few adult plants with only juvenile foliage (this is not uncommon in *Juniperus*), so the separation of *J. saxicola* from *J. barbadensis* with juvenile leaves, might not be

possible in the herbarium in this instance. *Juniperus virginiana* var. *virginiana* and *J. v.* var. *silicicola* are extremely similar in their morphology.

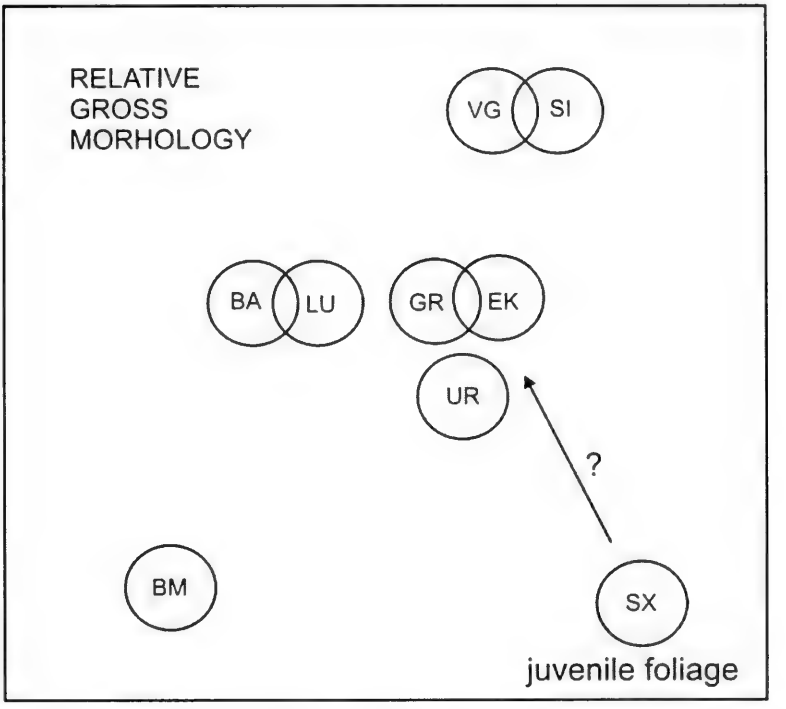

Figure 6. Relative gross morphology for the Caribbean junipers. Because *J. saxicola* (SX) has only juvenile leaves, it is appears very distinct, but it may relate to the typical morphology.

The data sets examined in this study agree in several facets: *J. bermudiana* (BM) is distinct; *J. v.* var. *virginiana* is distinct from the Caribbean junipers; all the other taxa are very similar. *Juniperus virginiana* var. *virginiana* and var. *silicicola* are very similar in all the analyses except the RAPDs and trnC-trnD SNPs. *Juniperus barbadensis* (BA) and *J. lucayana* (LU) are either identical or nearly identical in most analyses. This supports the recognition of *J.*

barbadensis var. *lucayana* (Adams, 1995, Farjon, 2005). Generally, the recognition of *J. gracilior* with two varieties (var. *ekmanii*, var. *urbaniana*) is supported by terpenoids, RAPDs and SNPs of nr DNA. The placement of *J. saxicola* is problematical. The terpenoids show it to be distinct (Fig. 2), somewhat similar to *J. lucayana* in Cuba, and the RAPDs show (Fig. 3) its affinity about equally to *J. barbadensis* and *J. lucayana*. The ITS SNPs place it (Fig. 4) intermediate between *J. gracilior* var. *urbaniana* and *J. barbadensis* - *lucayana* and the trnC-trnD SNPs place it with *J. g.* vars. *ekmanii* and *urbaniana*.

SPECIATION IN THE WEST INDIES

All of the junipers of the Caribbean have smooth-leaf margins (entire series), and no junipers from the denticulate (serrate) leaf-margined junipers (denticulate series) are present in the Caribbean. In contrast, the junipers found in southern Mexico and Guatemala are only in series denticulate (the southernmost range of *Juniperus* in the continental western hemisphere). The affinities of the Caribbean junipers are clearly not with the junipers of Central America. The spread of the junipers across the Caribbean islands has most likely been by birds from eastern North America (Fig. 7). The clearest evidence presented in this study is the trnC-trnD, 245 bp deletion in cp DNA that is shared by all Caribbean junipers, as well as *J. v.* var. *silicicola* and *J. bermudiana* (Fig. 6).

Colonization of *Juniperus* into the West Indies is postulated to have occurred by long distance bird dispersal of *J. virginiana* (or its ancestor) to Cuba or the Bahama Islands and then to Bermuda, Jamaica, and Hispaniola (Adams, 2004). *Juniperus saxicola* may have evolved from ancestral *J. gracilior*-like plants or from *J. barbadensis* var. *lucayana* plants of eastern Cuba from seeds carried into the Pico Turquino region. Either by a chance founder's effect or by genetic drift, the gene(s) for controlling the conversion from juvenile (awn-like) to adult (scale-like) leaves became fixed such that all adults now have only juvenile leaves. *Juniperus barbadensis* appears to have arisen from *J. barbadensis* var. *lucayana*. The large distance from Cuba to St. Lucia and the lesser Antilles render this hypothesis somewhat tentative. The alternative mode, island-hopping from Hispaniola may be a more attractive option, but at present, the lack of suitable habitat on many of the intervening islands makes this scenario unlikely.

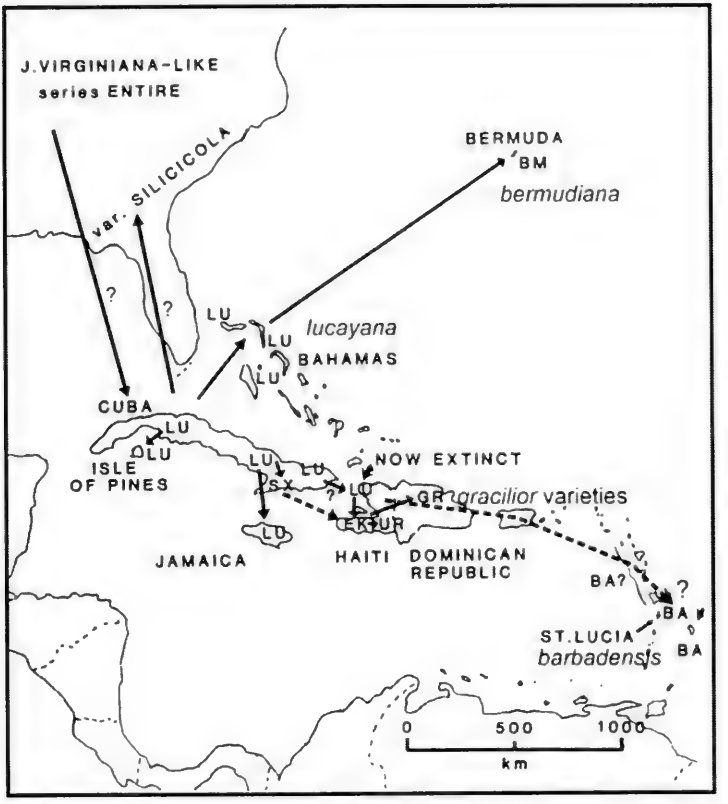

Figure 7. Postulated speciation of the smooth-leaf margined *Juniperus* of the Caribbean.

It seems plausible that the 245 bp deletion occurred in junipers in Cuba or Hispaniola and then this cpDNA type was introduced into *J. virginiana* in se USA to produce *J. v.* var. *silicicola*.

The junipers of Hispaniola appear to have arisen from *J. barbadensis* var. *lucayana* or its ancestor. Although *J. b.* var. *lucayana* seems now to be extinct in Hispaniola, specimens collected earlier in the twentieth century in northern Haiti appear to be *J. b.* var. *lucayana*. The junipers in the *J. gracilior* complex were likely derived from

ancestral *J. b.* var. *lucayana*. *Juniperus gracilior* var. *urbaniana* probably arose from *J. g.* var. *ekmanii* or its ancestor.

The introduction of *J. bermudiana* into Bermuda must have been relatively recent because Bermuda's soil was reportedly formed only during the first inter-glacial period of the Pleistocene (Bryan and Cady, 1934; Cox, 1959). Herwitz (1992) recently estimated the ages of the highest eolianite dunes on Bermuda (Southampton, 73 m elev.) at 85,000 yr bp and the oldest hill, Walsingham (29 m elev.) at greater than 880,000 yr bp.

Juniperus bermudiana, endemic to Bermuda, has been subject to attack by two scale insects, *Lepidosaphes newsteadi* and *Carulaspis minima*, that were apparently introduced from the U.S. mainland prior to 1942 (Bennett and Hughes, 1959; Groves, 1955). These insects cause defoliation and death. Groves (1955) estimated that 90% of the trees were dead by 1955. In 1978, William E. Sterrer, Bermuda Biological Station, (pers. comm.) estimated that perhaps 99% of the original trees were dead.

Considering the genetic bottleneck that the Bermuda junipers must have gone through in arriving at their current reduced state (Bennett and Hughes, 1959), we cannot be certain that extant trees fairly represent the gene pool that evolved on Bermuda. This may account in part, for the divergence of *J. bermudiana* from the Caribbean junipers.

The differentiation of these island populations has been affected both by selection and founders effects. Genetic drift may have also played a part in their diversification because of the expansion and contraction of their ranges during the Tertiary and Pleistocene. According to Curray (1965), the Caribbean sea level dropped approximately 122 m about 19,000 yr bp, with another drop in sea level of 146 m at 40,000 yr bp. Rosen (1978) showed that these drops in sea level would unite several of the Bahamian Islands. Conversely, a rise in the ocean level of only a few meters would inundate many juniper sites in the Bahamas where *J. lucayana* often occurs at 1 to 2 m above sea level. Broecker (1965) reported evidence for higher levels about 80,000 yr bp in the Bahamas. Thus, there is ample evidence of changes

in available juniper habitat, which in turn has probably led to local extinctions as well as range expansions. This, coupled with limited gene flow between the islands, has led to the considerable amount of diversity and differentiation in the Caribbean junipers.

Based on the data presented, the recognition of the taxa as shown in table 1 is prudent. Additional kinds of data may provide more insight into these relationships, but it seems likely that a new data set may not completely resolve these complex patterns.

Table 1. *Juniperus* of the Caribbean.

Taxon	Distribution, status
J. barbadensis var. *barbadensis*	St. Lucia, BWI, endemic, endangered
J. barbadensis var. *lucayana*	Cuba, Jamaica, Bahamas, threatened
J. bermudiana	Bermuda, endemic, endangered Threatened by hybridization with introduced *J. virginiana.*
J. gracilior var. *gracilior*	Dominican Republic, threatened?
J. gracilior var. *ekmanii*	Haiti, Dom. Rep., endangered
J. gracilior var. *urbaniana*	Haiti, endangered
J. saxicola	Cuba, Pico Turquino, endangered?
J. virginiana var. *virginiana*	e US, weedy, expanding its range
J. virginiana var. *silicicola*	coastal fore dunes, se US, may be threatened by beach development

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Jim Robbins with *Verbesina jimrobbinsii* B. L. Turner, **sp. nov.** at the type locality in Jalisco, Mexico. See Turner, "Overview of the section Platypteris of *Verbesina* (Asteraceae) and description of a new species" in this issue.